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Algae-Enriched Bacterial Community Composition Varies with Stress Response Patterns in Antarctic Algal Enrichment Cultures

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Abstract

Perennially ice-covered lakes in the McMurdo Dry Valleys, Antarctica, are shaped by permanent stratification, extreme oligotrophy, and salinity gradients, yet these features are vulnerable to climate-driven hydrologic change. Because phytoplankton and associated bacteria regulate carbon flow and nutrient cycling, understanding how algal–bacterial consortia respond to disturbance is key to predicting ecosystem change. We used enrichment cultures from Lakes Bonney and Fryxell to test responses to nutrient deprivation and salinity alteration, two perturbations relevant to climate-driven changes in hydrologic connectivity and expansion of open water moats. Autotrophic enrichments lacking added organic carbon were used to enrich algal–bacterial consortia dependent on photosynthetically derived substrates. Community responses were assessed with 16S rRNA amplicon sequencing of size-fractionated samples, allowing comparison of particle-associated and planktonic communities. Short-term nutrient limitation produced only limited shifts in community composition, indicating resistance to transient nutrient stress. However, bacterial communities were strongly structured by size fraction: particle-associated assemblages separated clearly from planktonic communities and were enriched in taxa linked to algal surfaces and polysaccharide-rich microhabitats, including *Flavobacteriales*, *Sphingobacteriales*, *Rhizobiales*, and *Rhodobacterales*. Salinity perturbation drove stronger restructuring of bacterial communities, with shallow Lake Bonney enrichments showing greater sensitivity than deeper communities. These findings suggest that algae-associated bacterial communities help structure Antarctic algal enrichment cultures and may influence microbial responses to climate-linked disturbance.

Keywords: McMurdo Dry Valleys; Antarctic lakes; phycosphere; algal–bacterial consortia; nutrient limitation; salinity stress; microbial community assembly; community resistance

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1. Introduction

Climate-driven environmental change is reshaping aquatic ecosystems by altering temperature regimes, mixing dynamics, and nutrient availability, with especially pronounced consequences in ecosystems structured by strong physical and chemical gradients [1,2]. In the perennially ice-covered lakes of the McMurdo Dry Valleys (Southern Victoria Land, Antarctica), hydrologic connectivity, moat formation, and changes in water-column mixing can expose otherwise stable microbial communities to shifts in salinity and nutrient supply that differ sharply from their native conditions [3]. Because allochthonous inputs are limited, phytoplankton are the dominant primary producers in these oligotrophic lakes and are highly sensitive to environmental stress [4]. Their responses to environmental

perturbation may therefore influence not only phytoplankton performance, but also the cycling of carbon and nutrients within these microbial-dominated food webs.

Phytoplankton do not live in isolation. The release of phytoplankton-derived photosynthetic exudates creates a microhabitat enriched in dissolved, simple organic compounds, termed the "phycosphere" [5]. Functionally, the phycosphere facilitates metabolite exchange between phytoplankton and bacteria, thereby recruiting taxa-specific bacterioplankton into the phycosphere habitat. Metabolic exchange between these two groups drives a host of interactions ranging from mutualistic to antagonistic [6]. Bacterial partners can promote phytoplankton growth by providing phytohormones, vitamins, and regenerated nutrients [7–10]. However, bacteria can also inhibit algal growth through competition or the release of algicidal compounds [11,12]. Likewise, the release of highly labile organic compounds by phytoplankton can stimulate increased growth of bacteria, while production of antibacterial compounds allows for defense by the phytoplankton in response to bacterial antagonism [13,14]. Together, these complex combinations of interactions within the phycosphere complicate the classical view of the microbial loop by positioning phytoplankton-associated bacteria not simply as consumers of algal carbon, but as active regulators of host physiology and even resilience to stress [15,16].

Perennially ice-covered lakes within the McMurdo Dry Valleys (MDV) represent compelling sites for studying microbial interactions under stress. These meromictic lakes are permanently stratified and exhibit minimal allochthonous inputs [17,18]. Lakes Bonney and Fryxell are oligotrophic systems in which photosynthetic protists contribute substantially to organic carbon production [19], with heterotrophic bacteria utilizing a large fraction of the DOC (dissolved organic carbon) released by phytoplankton [20,21]. Nutrient limitation is a defining feature in these lakes, constraining phytoplankton productivity: previous studies have shown that nitrogen and phosphorus availability can constrain phytoplankton growth [22]. Communities are also exposed to variable salinity through vertical separation within stratified water columns, and climate-driven meltwater inputs may also generate localized freshening, particularly near lake margins and peripheral moats. Together, these characteristics make MDV lakes well suited for testing how environmental perturbations alter algal–bacterial consortia.

Although the MDV lakes are described as stable, they have experienced climate-related changes, and their microbial communities are highly susceptible to environmental disturbances. Increasing air temperatures due to climate change have driven increased hydrological connectivity, lake level rise, and expansion of open water peripheral moats [23,24]. Seasonal and long-term climate-driven changes can influence nutrients, salinity, photosynthetically available radiation, and mixing regimes between moats and chemoclines [25,26]. Prior work has shown that microbial community structure within MDV lakes is significantly influenced by the physiochemical stratification within the lakes [18,26,27]. However, much less is known about how algal-associated bacterial communities respond to variability in key drivers, such as nutrient availability and salinity, or whether algal–bacterial associations contribute to the compositional stability of Antarctic enrichment communities under environmental stress.

In this study, we used enrichment cultures to examine how algal–bacterial consortia from McMurdo Dry Valley lakes respond to nutrient and salinity perturbations, key environmental drivers likely to change under future climate conditions. Long-term laboratory enrichment cultures were established from the chlorophyll maxima of East Lake Bonney, West Lake Bonney, and Lake Fryxell, representing communities originally derived from environments with contrasting nutrient and salinity conditions. Nutrient deprivation assessed resistance to reduced nitrogen and phosphorus availability, while salinity perturbation simulated shifts in ionic conditions associated with climate-driven hydrologic

change. We hypothesized that: (1) communities would show relative resistance to nutrient limitation because they originate from chronically nutrient-poor environments; (2) salinity shifts would more strongly affect shallow communities, which are less likely to be adapted to high-salt conditions; and (3) particle-associated bacterial communities would differ from planktonic communities, consistent with the formation of distinct algae-associated assemblages that may influence community responses to disturbance.

2. Materials and Methods

2.1. Site Description and Sample Collection

Samples were collected during the austral summers of 2020 and 2022 from ice holes within the centers of East Lake Bonney (ELB; 77°42′51.3″ S, 162°26′37.5″ E), West Lake Bonney (WLB; 77°43′12.4″ S, 162°17′52.1″ E), and Lake Fryxell (FRX; 77°36′37.7″ S, 163°08′43.9″ E), three meromictic lake basins located within Taylor Valley, McMurdo Dry Valleys. Representative geochemical profiles from each lake basin are shown in Figure 1A. Sampling depths were selected to target chlorophyll maxima identified from in situ fluorescence profiles (Figure 1B) measured with a FluoroProbe (BBE Moldaenke GmbH, Schwentinental, Germany). At each sampling depth, 5 L of water was collected using a 5-L Niskin bottle (General Oceanics). Samples were collected from 15 m in ELB, 6 and 15 m in WLB, and 9 m in FRX. Samples were stored at 4 °C in the dark until processing.

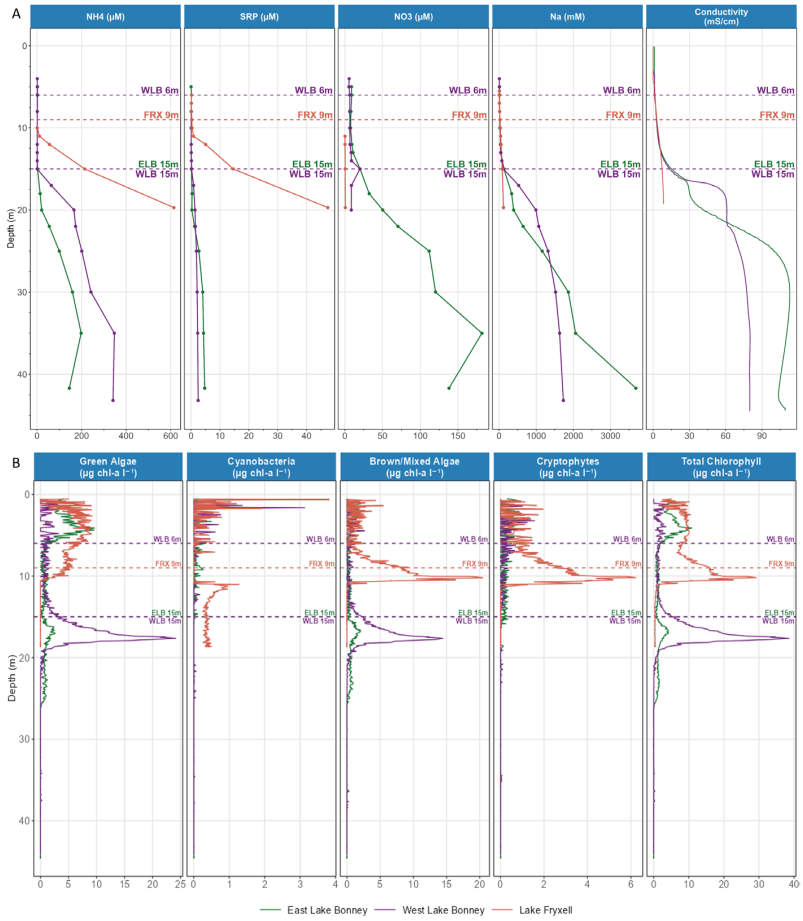

Figure 1. Environmental gradients and phytoplankton composition across source lakes. (**A**) Depth profiles of dissolved NH_4, SRP, NO_3, sodium, and conductivity in East Lake Bonney (green), West Lake Bonney (purple), and Lake Fryxell (orange). (**B**) Depth profiles of spectral algal classes estimated from chlorophyll-a fluorescence for the same lakes. Dashed lines denote sampling depths. Colors are consistent between panels. Dotted lines indicate sampling depths where water was collected for enrichment culturing.

2.2. Enrichment Cultivation

Lake water samples were amended with L1 growth medium (Bigelow NMCA, East Boothbay, ME, USA) prior to transport to Miami University. This enrichment strategy was designed to favor photosynthetic protists while also supporting associated heterotrophic bacteria through algal release of photosynthetically derived organic substrates. Because the medium contains no exogenous organic carbon source, bacterial growth is expected to depend largely on algal primary production. Enrichment samples were maintained in 125-mL flasks and incubated with shaking at 8 °C under 50 µmol m^{-2} s^{-1} of light to approximate conditions that would be found within the lakes.

Samples obtained from ELB and FRX during the 2020 austral summer (Table S1) were supplemented with 10× L1 growth medium to a final concentration of 0.5× L1. Enrichments were grown in a temperature-regulated photoincubator in McMurdo Station prior to shipment at 4 °C. Upon arrival in the United States, cultures were maintained by monthly transfer of 10% inoculum into fresh medium (0.5× L1, 50% seawater) under the growth/media conditions (Table S1). This approach was previously established to promote the growth of diverse MDV algal species [28]. These enrichments were subsequently used in the nutrient deficiency experiments.

Samples collected from WLB during the 2022 austral summer were obtained from two depths (6 m and 15 m; Table S1). Enrichments were established as described above, except enrichments were maintained at multiple salinity treatments in addition to supplementation with 10× L1 medium to a final concentration of 0.5× L1. These growth conditions were continued during monthly passages (Table S1). These enrichments were then used for subsequent salinity stress experiments.

2.3. Nutrient Limitation Bioassay

For nutrient perturbation experiments, enrichment cultures were first stabilized through four generations of subculturing at a 1:10 inoculum-to-medium ratio. Cultures were then concentrated using tangential flow filtration with a 0.45-µm Pellicon XL filter (Millipore, Burlington, MA, USA) and washed with nutrient-free L1 medium lacking both nitrogen and phosphorus. Washed enrichments were inoculated in triplicate into 50-mL culture flasks containing control L1 medium, nitrogen-limited L1 medium (0.95 µM N; L1-N), phosphorus-limited L1 medium (0.03 µM P; L1-P), or L1 medium lacking both nitrogen and phosphorus (L1-NP). Nutrient-limited conditions were selected based on nitrogen and phosphorus concentrations measured in the nutrient-limited shallow depths of Lakes Bonney and Fryxell [29]. Cultures were incubated at a temperature/light regime of 8 °C/50 µmol m^{-2} s^{-1} with the flask lids loosened to permit gas exchange during the four-week incubation period. Samples were collected immediately prior to treatment inoculation and then twice weekly during the four-week incubation. For amplicon sequencing, samples were obtained from three independent biological replicate cultures at inoculation (T0), week 1 (T1), and week 4 (T4). These samples represent repeated measurements from the same cultures over time. Each culture was maintained at a final volume of 20 mL throughout the experiment.

2.4. Salinity Bioassay

Salinity perturbation experiments were conducted using WLB enrichment cultures initiated during the 2022 austral summer. Prior to treatment, enrichments from 6 m and 15 m were passaged for four generations to stabilize community composition under laboratory conditions. Cultures were then inoculated into 125-mL flasks containing L1 media supplemented with NaCl to final concentrations of 200- (low salt), 500- (medium salt), and 750-mM (high salt) NaCl. Shallow cultures were excluded from high salt supple-

mentation, as pre-screening indicated complete culture death under the 750-mM NaCl supplementation. To simulate freshening (FR) of the water column from meltwater input or mixing, a subset of the enrichments was inoculated into L1 media diluted to a final seawater concentration of 25%. Cultures were incubated at 8 °C under 50 µmol m^{-2} s^{-1} of light for three weeks, after which samples were collected for community analysis.

2.5. DNA Extraction and Amplicon Sequencing

Samples for community analysis were fractionated by sequential filtration through a 5-µm polycarbonate filter to capture the particle-associated fraction, followed by filtration of the flow-through onto a 0.1-µm filter to capture the planktonic fraction. DNA was extracted from cut filter pieces, using a FastDNA spin kit for soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. For the nutrient bioassay, amplicon sequencing was conducted on samples from three independent biological replicate cultures collected at inoculation (T0), week 1 (T1), and week 4 (T4), representing repeated measurements over time. No technical replicates were included. The 16S rRNA V4 region was amplified using primers 515F/806R, and the 18S rRNA V9 region was amplified using primers 1391F/1501R, each with barcodes and linker sequences [30–32]. Amplicon libraries were prepared following the Earth Microbiome Project protocols (https://earthmicrobiome.org/) and sequenced in-house using a 500-cycle MiSeq Reagent Kit V2 (Illumina, San Diego, CA, USA) on a MiSeq platform with a 2 × 250 bp chemistry and 10% PhiX spike-in [33].

2.6. Sequence Processing and Community Analyses

Demultiplexed 16S and 18S amplicon reads were processed in R v4.3.3 using the DADA2 pipeline to infer amplicon sequence variants (ASVs) within the RStudio (v. 2025.05) environment [34–36]. Bacterial ASVs were classified against SILVA v138.2, and eukaryotic ASVs were classified against PR2 v5.0.1. ASV tables were rarefied to 3000 reads per sample using the rarefy_even_depth function in phyloseq [37–39]. Alpha diversity metrics, including Shannon diversity and Chao1 richness, were calculated in microeco (v. 1.10.0, [40]). Differences in alpha diversity between filter fractions were evaluated using Wilcoxon rank-sum tests, whereas effects of nutrient treatment, salinity treatment, depth, and culture type were assessed using analysis of variance. Beta diversity was assessed using Bray–Curtis dissimilarity [41] and principal coordinate analysis (PCoA) was used to visualize differences in community structure across samples [42]. Differences in community composition among treatments, size fractions, depths, and culture types were tested using PERMANOVA based on Bray–Curtis dissimilarity. Linear discriminant analysis effect size (LEfSe) was employed to identify differentially abundant taxa between treatments [43]. 18S rRNA gene sequencing was not performed for the salinity bioassay.

3. Results and Discussion

3.1. Site Description

The enrichment cultures used in this study were derived from chlorophyll maxima located within distinct physicochemical environments across three McMurdo Dry Valley lakes. Source depths differed in nutrient availability, conductivity, and sodium concentrations, reflecting the strong vertical and among-lake gradients that characterize East Lake Bonney, West Lake Bonney, and Lake Fryxell (Figure 1A). FluoroProbe profiles further showed that sampling depths coincided with phytoplankton biomass maxima or distinct algal assemblages within each lake basin (Figure 1B). Because enrichment cultures were generated from different lake basins, depths, and field seasons, these source conditions provide important context for interpreting treatment responses. Nutrient-deprivation experiments were conducted using 2020 enrichments from ELB and FRX, whereas salinity

perturbation experiments used 2022 shallow and deep WLB enrichments, allowing us to test stress responses across communities originating from contrasting environmental settings (Table S1).

3.2. Nutrient Limitation Has Limited Effects on Community Structure

To assess how nutrient limitation influenced enrichment community composition, we analyzed taxonomic abundance and diversity patterns of both the prokaryotic and eukaryotic communities. After quality filtering, 840,095 16S rRNA gene reads and 1,770,543 18S rRNA gene reads were retained, corresponding to 429 bacterial and 1616 eukaryotic ASVs, respectively. Bacterial communities were dominated by taxa affiliated with *Proteobacteria*, *Bacteroidota*, and *Actinomycetota*, which accounted for approximately 80% to 99% of the total bacterial relative abundance across all cultures (Figure 2A–C). Eukaryotic communities were predominantly comprising green algae in the order Chlorellales, which was the most abundant across all cultures, averaging over 45% of the total relative abundance (Figure S1). Nutrient-limited conditions did not significantly alter prokaryotic (Figure 3A–C) or eukaryotic (Figure S3) community composition based on Bray–Curtis dissimilarity of 16S and 18S rRNA gene amplicon communities. Similarly, nutrient treatments did not produce clear shifts in dominant bacterial (Figure 2A) or eukaryotic taxa based on relative abundance patterns (Figure S3). These results suggest that algal–bacterial enrichment communities derived from McMurdo Dry Valley lakes were relatively resistant to short-term nitrogen and phosphorus limitation under the conditions tested.

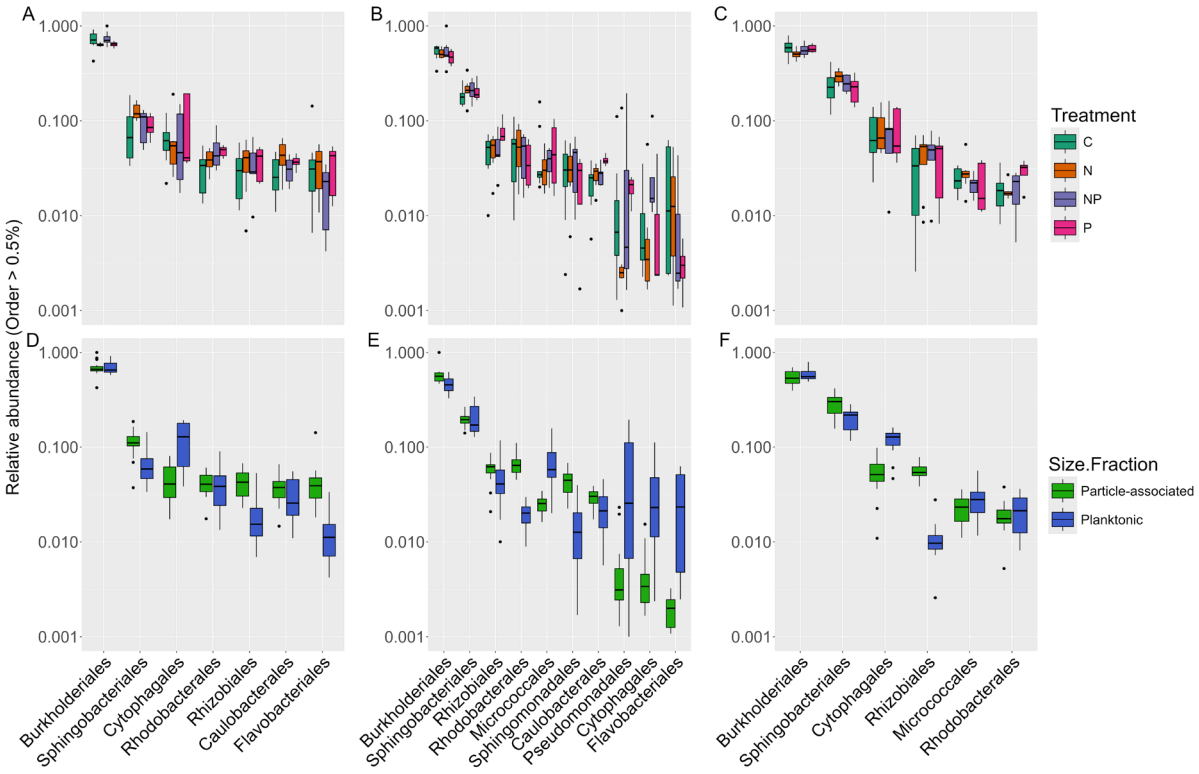

Figure 2. Nutrient limitation and size fraction alter bacterial community composition in Antarctic enrichment cultures. The relative abundance of 16S rRNA amplicon sequences at the order level, with cultures subdivided by treatment conditions (**A**–**C**) and size fraction (**D**–**F**) with shown taxa representing orders with greater than 0.5% relative abundance. Black dots represent individual samples.

The limited compositional response of enrichment communities to nutrient deprivation contrasts with prior nutrient-amendment studies in several MDV lakes, which have shown that natural planktonic communities can be highly responsive to additions of ni-

trogen and phosphorus. Priscu [22] reported lake-specific patterns of nutrient deficiency, including strong phosphorus deficiency in Lake Bonney and nitrogen deficiency in Lakes Hoare and Fryxell, with combined nitrogen and phosphorus amendments stimulating photosynthesis in some lake communities. More recently, Teufel et al. [21] showed that inorganic nitrogen and phosphorus availability can influence phytoplankton production and bacterial community structure in oligotrophic upper waters of Lakes Bonney and Fryxell, likely through nutrient-driven changes in autochthonous carbon production and availability. In contrast, the enrichment cultures examined here showed limited shifts in bacterial or eukaryotic community composition under short-term nutrient deprivation, suggesting that these cultures may represent algal–bacterial consortia with relatively high resistance to nutrient stress.

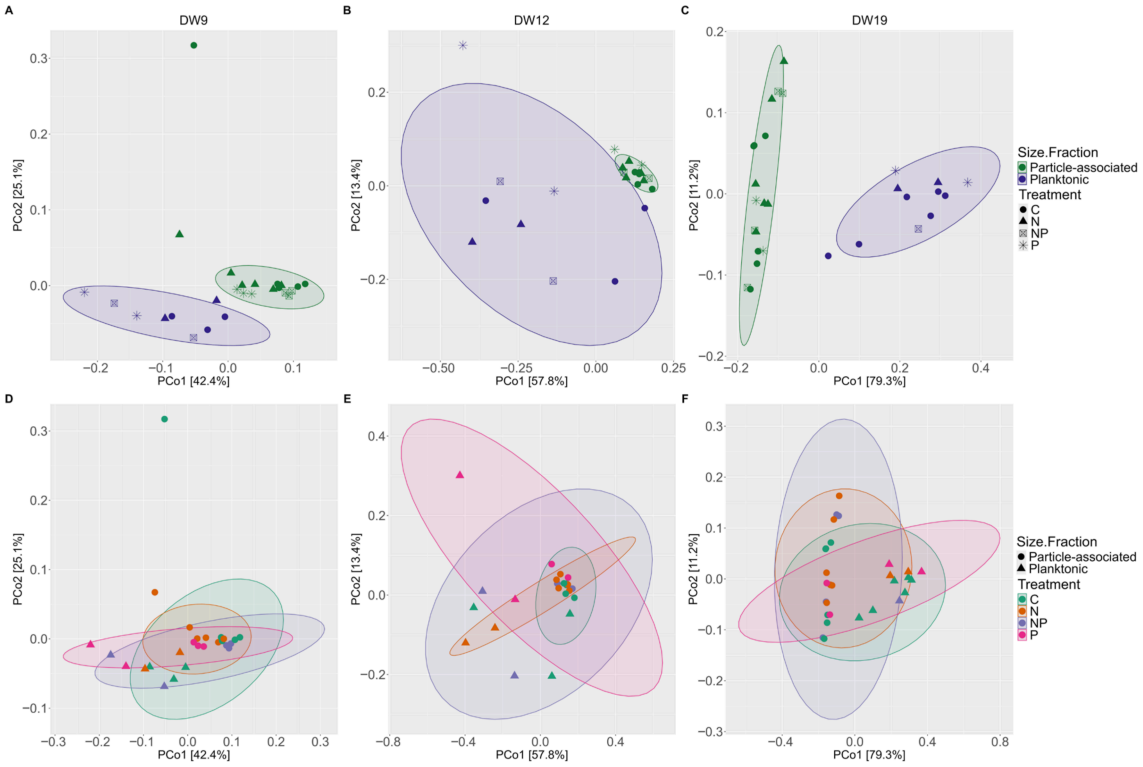

Figure 3. Community composition under nutrient limitation separates primarily by size fraction, with secondary effects of treatment and culture identity. Effects of nutrient limitation (**A**–**C**) and size fractionation (**D**–**F**) on bacterial communities. Bray–Curtis dissimilarity of the 16S rRNA communities calculated at the ASV level.

The apparent stability of these enrichment communities under nutrient limitation is consistent with the chronic nutrient-poor conditions that characterize McMurdo Dry Valley lakes. It may also reflect metabolic interactions within algal–bacterial consortia, including nutrient recycling or exchange within the phycosphere, although these processes were not directly tested in this study. Previous studies have shown that phycosphere-associated bacteria can influence algal responses to nitrogen and phosphorus imbalance while maintaining their own persistence through streamlined bacterial genomes and altered macronutrient metabolism genes [44,45]. Furthermore, in this study, members of *Rhizobiales* and *Rhodobacterales* were detected across all cultures, and related bacterial taxa have been implicated in nitrogen exchange with phytoplankton in other aquatic systems [10]. However, because our study used amplicon sequencing rather than direct functional measurements, the mechanisms underlying community stability cannot be directly resolved. Together, these results suggest that algal-associated bacterial communities may contribute

to resistance under nutrient-limited conditions, but additional functional assays would be needed to test this directly.

3.3. Impact of Salinity Stress on Bacterial Community Diversity and Structure

Salinity is a major driver of microbial community structure and function in aquatic ecosystems. Increases in salinity can reduce the growth and productivity of phytoplankton by disrupting ion homeostasis, increasing osmotic stress, and promoting the accumulation of reactive oxygen species, which may reduce photosynthetic activity and carbon fixation [46]. Salinity shifts can also directly alter bacterial community diversity, composition, and structure, particularly in freshwater or stratified aquatic ecosystems exposed to salt intrusion or altered mixing regimes [47,48].

To evaluate how salinity perturbation affected bacterial communities within Antarctic algal enrichment cultures, we investigated shallow and deep WLB enrichments derived from 6 m and 15 m, respectively, representing microbial communities exposed to significantly different salt concentrations (Figure 1A). Our treatments were designed to simulate climate-relevant salinity changes that could occur during either increased glacial stream intrusion or altered mixing across the water column. Although 18S rRNA gene sequencing was not performed for the salinity bioassay, plastid 16S rRNA sequences indicated that the algal component of the WLB enrichments was dominated by chlorophyte sequences across treatments (Figure S5). Thus, the salinity bioassay is best interpreted as a bacterial community response within chlorophyte-dominated algal enrichment cultures, rather than as a fully resolved analysis of parallel bacterial and eukaryotic community change.

After quality filtering, 1,755,641 16S rRNA gene reads were retained and assigned to 780 ASVs. Across salinity treatments, bacterial communities were dominated by *Bacteroidota* and *Proteobacteria*, which together represented approximately 80–95% of total reads. These phyla were primarily represented by taxa affiliated with *Flavobacteriales*, *Rhizobiales*, and *Burkholderiales* (Figure 4). Salinity treatment strongly influenced the structure of shallow enrichment communities, with control and low-salt treatments clustering separately from the other treatments based on Bray–Curtis dissimilarity (PERMANOVA, $p < 0.01$, Figure 5A). Freshening and medium-salt treatments showed partial overlaps in shallow enrichments, suggesting that these treatments selected for similar bacterial community structures despite representing different salinity perturbations. This overlap appeared to be associated with similarities in dominant bacterial taxa across these treatments (PERMANOVA, $p < 0.05$, Figure 4A).

In contrast, compositional changes in deep enrichment communities in response to salt amendments were more subtle. Freshening, medium-salt, and high-salt treatments showed greater overlap in ordination space (PERMANOVA, $p < 0.05$, Figure 5B), whereas low salt treatments clustered separately from the other treatment conditions. These trends suggest that bacterial communities derived from shallow WLB enrichments were more responsive to salinity perturbation than those derived from deeper enrichments.

The depth-dependent response is consistent with previous in situ work showing greater sensitivity of near-surface Lake Bonney microbial communities to climate-relevant disturbance. Priscu [22] observed that the productivity of shallow communities collected from ELB was stimulated by the addition of nutrients, while communities from the chemocline responded minimally to nutrient addition. Likewise, Sherwell and colleagues [49] simulated lake-level rise and moat expansion by transplanting dialysis-bagged communities across the water column and into peripheral moat conditions, and found that communities originating from 5 m surface waters shifted in both bacterial and eukaryotic composition, whereas communities from deeper waters were comparatively less affected. In the present study, shallow WLB enrichments similarly showed stronger treatment-driven separation

under salinity perturbation, whereas deep enrichments showed more muted compositional responses. However, because 18S rRNA gene sequencing was not performed in the salinity bioassay, bacterial community responses cannot be interpreted independently of potential eukaryotic (algal) community shifts, which may also respond to salinity and contribute to observed community restructuring. Together, these results suggest that shallow MDV lake communities may be especially vulnerable to climate-driven changes that alter water-column position, salinity exposure, and connectivity with moat environments.

The reduced response of deep enrichment communities to elevated salt amendments may reflect greater tolerance of communities originating from deeper, more saline regions of the lake. Past studies on Lake Bonney phytoplankton have clearly shown that *Chlamydomonas* spp. isolated from deeper in the water column possess high salinity tolerance, relative to those from shallow depths [50]. Previous studies have shown that algal-associated bacteria can influence algal aggregation, osmolyte production, or extracellular polymeric substance formation under salt stress [51], raising the possibility that bacterial community composition contributes to differences in salinity response; however, this remains untested here. Additional physiological, metagenomic, or transcriptomic analyses would be needed to determine whether these bacterial taxa actively mediate salt tolerance in Antarctic algal–bacterial consortia. Importantly, our experimental salinity amendments (NaCl and sea-water additions) represent simplified proxies for salinization and do not fully reflect the complex ionic composition of Lake Bonney. Instead, our treatments approximate increases in total salinity rather than replicating full in situ geochemical conditions. Therefore, our results should be interpreted in the context of general osmotic stress responses rather than exact in situ geochemical shifts.

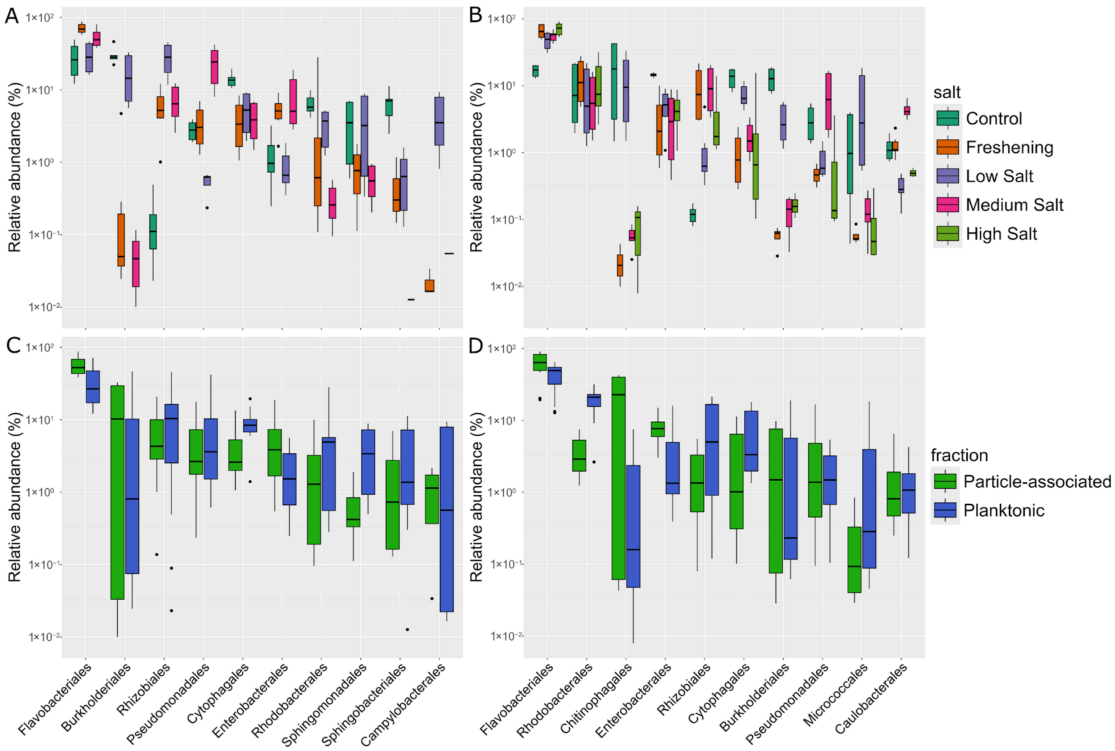

Figure 4. Salinity perturbation and size fraction reshape bacterial community composition in shallow and deep West Lake Bonney enrichments. Effects of size fractionation and salt amendment on the abundance of bacterial communities from shallow (**A**,**C**) and deep (**B**,**D**) enrichment cultures. The relative abundance of 16S rRNA amplicon sequences is shown at the order level, with cultures subset first by salinity (**A**,**B**) and size fraction (**C**,**D**).

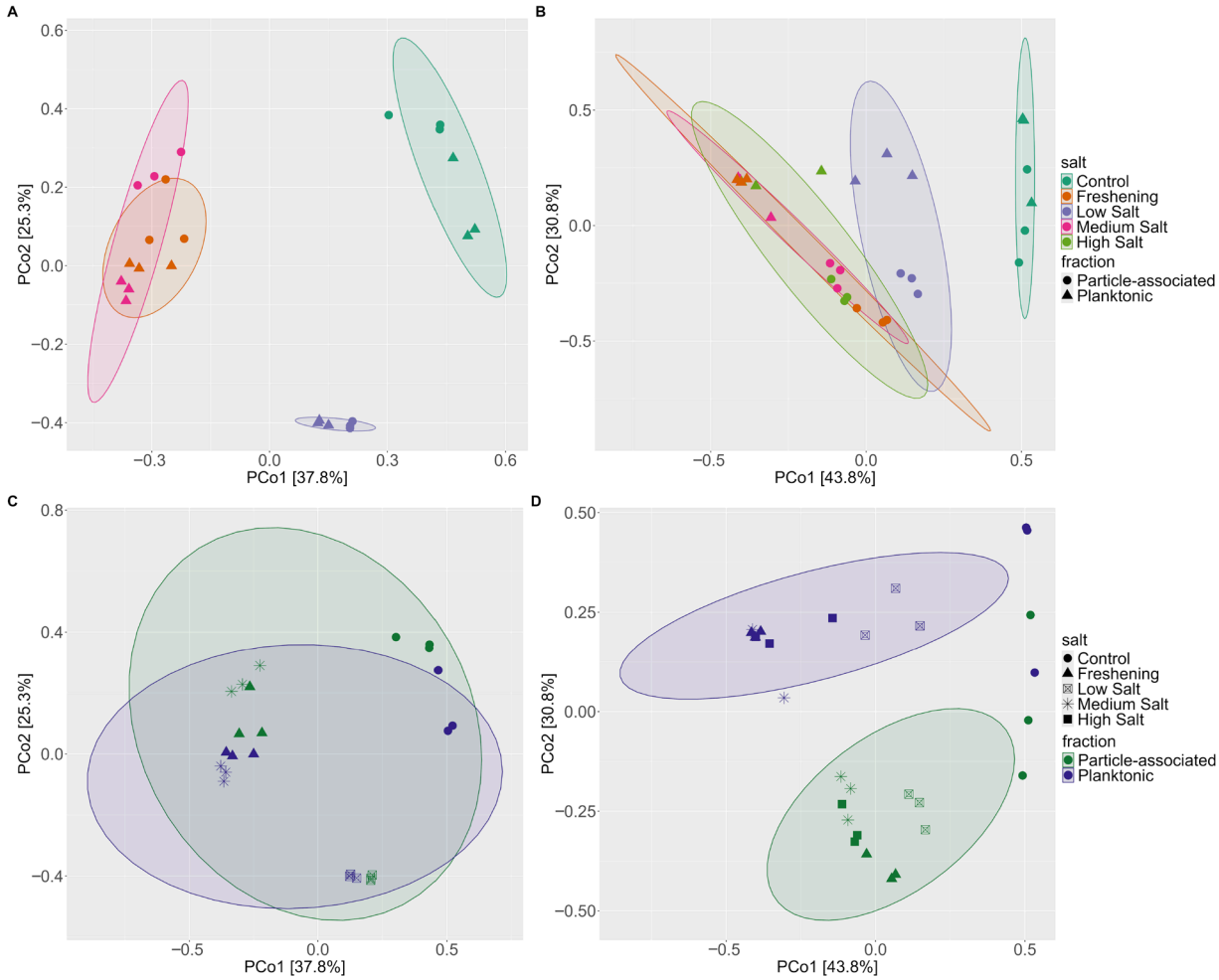

Figure 5. Bacterial communities respond to salinity perturbation in a depth- and fraction-dependent manner. Effects of salt amendment (**A**,**B**) and size fractionation (**C**,**D**) on shallow (**A**,**C**) and deep (**B**,**D**) bacterial communities. Principal coordinate analysis of Bray–Curtis dissimilarity of the 16S rRNA communities calculated at the ASV level.

3.4. Size Fraction Reveals Distinct Bacterial Communities Associated with Algal Particles

Bacteria associated with algae within the phycosphere can affect algal physiology, growth, and stress tolerance through metabolite exchange, nutrient remineralization, vitamin production, and modification of the local chemical environment within the phycosphere [44,45,51]. These interactions are expected to be strongest among bacteria in close physical association with algal cells or algal-derived particles. Therefore, we compared particle-associated and planktonic fractions to identify potential bacterial taxa that may be more closely linked to algal hosts or algal-derived organic matter.

Using a size-fractionation approach combined with amplicon sequencing, we evaluated the differences between particle-associated and planktonic microbial communities in response to nutrient limitation and salt stress. Under nutrient-limited and control conditions, bacterial communities consistently separated by size fraction across enrichment cultures, with particle-associated communities clustering separately from planktonic communities based on Bray–Curtis dissimilarity (PERMANOVA, $p < 0.001$; Figure 3D–F). In contrast, eukaryotic communities did not show strong separation by size fraction, with particle-associated and planktonic fractions clustering together across cultures (Figure S2). This suggests that size fractionation primarily resolved differences in bacterial community organization rather than major differences in eukaryotic community composition.

Differential abundance analysis further supported distinct bacterial membership between particle-associated and planktonic fractions. In Lake Bonney enrichment cultures (DW9/DW19), particle-associated fractions were enriched in *Sphingobacteriales*, *Flavobacteriales*, and *Rhizobiales*, whereas planktonic fractions were associated primarily with *Cytophagales* (LEfSe, LDA > 3; Figure 6A,C). In the Lake Fryxell enrichment culture DW12, particle-associated fractions were enriched in Rhodobacterales and *Sphingomonadales* (LEfSe, LDA > 3; Figure 6B). Several of these enriched groups, including *Cytophagales*, *Flavobacteriales*, and *Sphingobacteriales*, are associated with *Bacteroidota*, a phylum commonly enriched in the phycosphere due to its members' capacity to degrade complex polysaccharides [52,53]. Their enrichment in particle-associated fractions is consistent with the interpretation that algal particles and algal-derived substrates structure bacterial community assembly within these enrichment cultures.

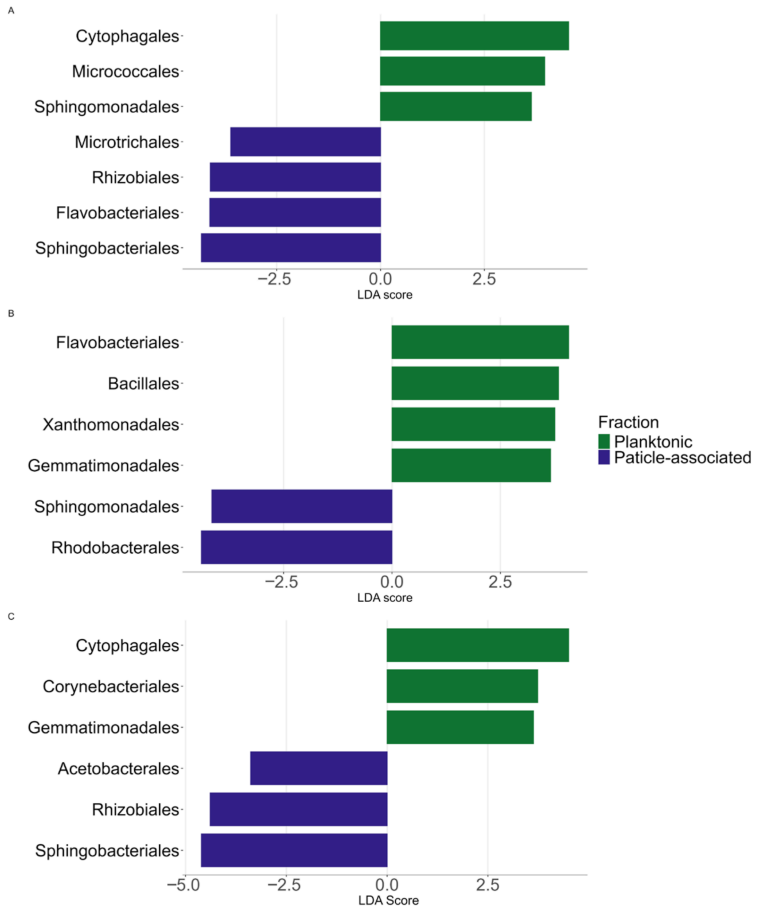

Figure 6. Distinct bacterial orders differentiate particle-associated and planktonic fractions across enrichment cultures. Linear discrimination analysis effect size (LEfSe, $p < 0.01$) of bacterial orders driving differences in Bray–Curtis dissimilarity matrices across all samples for each culture. Cultures DW9 (**A**) and DW19 (**C**) from East Lake Bonney and DW12 (**B**) from Lake Fryxell are divided into particle-associated (green) and planktonic (blue) groups discerning the representative orders.

The enrichment of *Rhizobiales*, *Rhodobacterales*, and *Sphingomonadales* in particle-associated fractions may also be ecologically relevant because related taxa are known to participate in nutrient exchange, organic matter transformation, and close associations with phytoplankton in aquatic systems [10,54]. These patterns are consistent with the possibility that particle-associated bacteria contribute to nutrient recycling within algal–bacterial consortia, potentially helping explain the limited compositional response to nutrient deprivation observed in these cultures. However, because these inferences are based on taxonomic composition alone, functional measurements would be required to determine

whether these taxa actively contributed to nitrogen regeneration, phosphorus recycling, or other nutrient-exchange processes.

Size fractionation also influenced bacterial community structure in the salinity bioassay, but the strength of this effect differed between shallow and deep WLB enrichments. In shallow enrichments, size fraction had a significant but relatively modest influence on community structure (Figure 5C,D), with planktonic fractions enriched in *Cytophagales* and *Sphingomonadales* and particle-associated fractions enriched in *Flavobacteriales* and *Enterobacterales* (LEfSe, LDA > 4.5; Figure 7A). In contrast, size fraction was a stronger determinant of bacterial community structure in deep enrichments (PERMANOVA, $p < 0.001$, Figure 5D). In these cultures, planktonic fractions were enriched in Rhodobacterales, *Rhizobiales*, *Cytophagales*, and *Micrococcales*, whereas particle-associated fractions were enriched in *Flavobacteriales* and *Enterobacterales* (LEfSe, LDA > 4, Figure 7B).

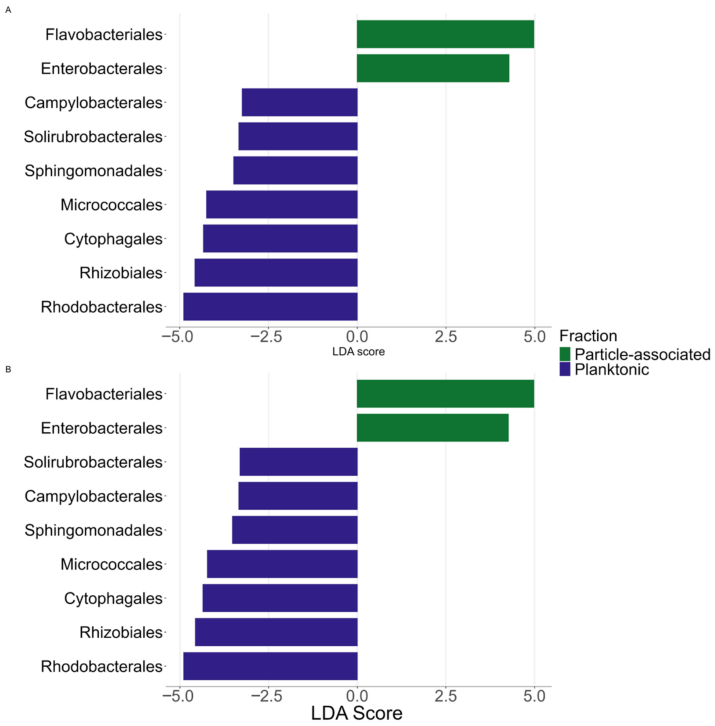

Figure 7. Particle-associated and planktonic fractions exhibit distinct bacterial orders across enrichment cultures. Linear discrimination analysis effect size (LEfSe, $p < 0.01$) of bacterial orders driving differences in Bray–Curtis dissimilarity matrices across all samples for each culture. Shallow (**A**) and Deep (**B**) enrichment cultures are divided into the particle-associated (green) and planktonic (blue) groups discerning the representative orders.

Together, these results suggest that particle-associated and planktonic bacterial communities represent distinct ecological fractions within algal enrichment cultures derived from Antarctic lakes. The relative importance of size fraction depended on both experimental context and source depth: shallow WLB communities were more strongly structured by salinity treatment, whereas deep WLB communities were more strongly structured by particle association. Deep enrichments were primarily structured by size fraction, whereas shallow enrichments were more strongly influenced by salt stress (Figure 5A,B). This inverse pattern suggests that bacterial lifestyle or attachment state may be especially important in deeper communities, while shallow communities may be more responsive to salinity perturbation. Differences observed among size-fractionated samples align with prior work demonstrating distinct community assembly in particle-associated versus planktonic bacterial communities, both in the MDV and other aquatic systems [18,55]. Lastly, the

observed differences between shallow and deep communities are consistent with previous studies identifying salinity as a major driver of bacterial community structure [49,55,56]. These patterns were observed within long-term algal–bacterial enrichment cultures derived from Antarctic lakes and therefore reflect responses within a laboratory-maintained system rather than in situ lake communities. Future studies incorporating in situ lake samples will be important to assess the extent to which these results reflect natural community structure.

4. Conclusions

Climate-driven changes in McMurdo Dry Valley lakes are expected to alter salinity, water column mixing, and nutrient availability, with consequences for microbial communities adapted to the highly stratified and historically stable conditions [3,57,58]. Taken together, our results suggest that algal–bacterial enrichment communities derived from Antarctic lakes exhibit stressor- and source-dependent responses to environmental perturbation. In contrast with previous studies reporting strong responses to nutrient addition, nutrient deprivation produced limited compositional changes. In the salinity experiment, salinity perturbation was associated with compositional shifts that differed between depths, with shallow communities showing stronger responses than deep communities. However, interpretation of salinity-driven bacterial restructuring is limited by the lack of eukaryotic community data, which may also respond to salinity and influence bacterial dynamics.

Across both experiments, size fractionation revealed distinct particle-associated and planktonic bacterial communities, highlighting the importance of close-proximity algal–bacterial associations in structuring enrichment communities. Our findings suggest that both environmental gradients and phycosphere-scale interactions may shape how MDV lake microbial communities will respond to future climate-driven change. Further work integrating physiological measurements, functional assays, and time-resolved community analyses will be needed to determine the mechanisms underlying this apparent resistance and to assess whether these patterns persist in more diverse natural lake communities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/phycology6030071/s1, Table S1. Summary of enrichment culture metadata, including isolation year, source lake, isolation depth, enrichment conditions, and treatment conditions of all cultures presented in this study. Figure S1. Nutrient limitation and size fraction alter bacterial and eukaryotic community composition in Antarctic enrichment cultures. Bacterial (a) and eukaryote (b) abundance under control (C), nitrogen-limited (N), phosphorus-limited (P), and nitrogen- and phosphorus-limited (NP) conditions. The relative abundance of 16S and 18S rRNA amplicon sequences is shown at the order level, with cultures subdivided into size fractions and treatment conditions with "Others" representing unclassified taxa or those under 0.1% of total relative abundance. Figure S2. Eukaryotic community composition under nutrient limitation separates primarily by culture identity, with secondary effects of treatment and size fraction. Effects of nutrient limitation (A–C) and size fractionation (D–F) on eukaryotic communities. Bray–Curtis dissimilarity of the 18S rRNA communities calculated at the ASV level. Figure S3. Alpha-diversity metrics of size-fractionated enrichment communities under nutrient-limited and control conditions. Prokaryotic (A,B) and eukaryotic (C,D) communities were analyzed across all cultures and treatments to evaluate differences in richness (A,C) and evenness (B,D) between particle-associated (green) and planktonic (blue) fractions (ANOVA, $p < 0.01$). Figure S4. Salinity perturbation and size fraction reshape bacterial community composition in shallow and deep West Lake Bonney enrichments. Effects of size fractionation and nutrient limitation on the abundance of bacterial communities from shallow (A) and deep (B) enrichment cultures. The relative abundance of 16S rRNA amplicon sequences is shown at the order level, with cultures subset first by salinity and further subset based on filter size with "Others" representing unclassified taxa or those under 0.1% of total relative abundance. Figure S5. Salinity amendments and size fraction alter plastid community composition in Antarctic

enrichment cultures. Eukaryotic plastid abundance under control, freshening, low salt (200-mM NaCl), medium salt (500-mM NaCl), and high salt (750-mM NaCl) amended conditions. The relative abundance of extracted plastid 16S rRNA amplicon sequences is shown at the division level, with cultures subdivided into 6-m (shallow) and 15-m (deep) enrichments, treatment conditions, and size fraction.

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Abbreviations

The following abbreviations are used in this manuscript:

MDV	McMurdo Dry Valleys
WLB	West Lake Bonney
ELB	East Lake Bonney
DOC	Dissolved Organic Carbon
PCoA	Principal coordinate analysis
LEfSe	Linear discriminant analysis effect size
ASV	Amplicon sequence variant
rRNA	Ribosomal RNA
LDA	Linear discriminant analysis

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