Depth and location influence prokaryotic and eukaryotic microbial community structure in New Zealand fjords

Sven P. Tobias-Hünefeldt[1], Stephen R. Wing[2],

Nadjejda Espinel-Velasco[2], Federico Baltar[2,3,*] and Sergio E. Morales[1,*]

[1]Department of Microbiology and Immunology, University of Otago, PO Box 56, Dunedin 9054, New Zealand

[2]Department of Marine Science, University of Otago, PO Box 56, Dunedin 9054, New Zealand

[3]Department of Limnology and Oceanography, Division of Bio-Oceanography, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

[*] **Corresponding authors:**

Dr. Sergio E. Morales, University of Otago, Department of Microbiology and Immunology, 720 Cumberland Street, North Dunedin, Dunedin 9054, New Zealand

Email: sergio.morales@otago.ac.nz, Phone: + 64 3 479 3140

Dr. Federico Baltar, Department of Limnology and Oceanography, Division of Bio-Oceanography, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Email: federico.baltar@univie.ac.at, Phone: +43-(0)1-4277-76436

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Summary

Systems with strong horizontal and vertical gradients, such as fjords, are useful models for studying environmental forcing. Here we examine microbial (prokaryotic and eukaryotic) community changes associated with the surface low salinity layer (LSL) and underlying seawater in multiple fjords in Fiordland National Park (New Zealand). High rainfall (1200-8000 mm annually) and linked runoff from native forested catchments results in surface LSLs with high tannin concentrations within each fjord. These gradients are expected to drive changes in microbial communities. We used amplicon sequencing (16S and 18S) to assess the impact of these gradients on microbial communities and identified depth linked changes in diversity and community structure. With increasing depth we observed significant increases in Proteobacteria (15%) and SAR (37%), decreases in Opisthokonta (35%), and transiently increased Bacteroidetes (3% increase from 0 to 40 m, decreasing by 8% at 200 m). Community structure differences were observed along a transect from inner to outer regions, specifically 25% mean relative abundance decreases in Opisthokonta and Bacteroidetes, and increases in SAR (25%) and Proteobacteria (>5%) at the surface, indicating changes based on distance from the ocean. This provides the first in-depth view into the ecological drivers of microbial communities within New Zealand fjords.

1. Introduction

Microorganisms play a major role in the cycling of both organic and inorganic nutrients in all major ecosystems (Falkowski et al., 2008; Junge et al., 2006; Sullam et al., 2017; Zahran, 1999). In particular, marine microbes have been linked to global nutrient cycling with community changes affecting global balances of carbon and nitrogen (Arrigo, 2005). Further, as both primary producers and recyclers, they provide information about ecosystem health, viability, and function (Azam and Worden, 2004; Graham et al., 2016). It is therefore critical to understand the factors controlling the microbial community structure in the marine environments. Up to date, a wide range of marine ecosystems remains understudied, including nearshore systems such as fjords. Indeed, fjord-systems with strong horizontal and vertical environmental gradients can be extremely useful models for studying the response of microbial communities to environmental factors.

Fjords are long narrow coastal inlets flanked by steep cliffs, typically carved by glaciers and resulting in deep waterways. In New Zealand (NZ), these sites receive large amounts of rain (>400 mm per month) (NIWA, 2016) and carbon per unit area (Smith et al., 2015) through exogenous carbon inputs particularly tannins (Currey et al., 2009). The external freshwater inputs help create vertical and horizontal gradients along the transition towards inland from the sea, and with depth, consistent with other sites (Storesund et al., 2017). These gradients can differ based on geographic (i.e. catchment area size) and environmental (i.e. land cover) variables resulting in highly diverse marine, and microbial communities, even in relatively close geographical locations (Brattegard, 1980; Herlemann et al., 2011). Microbial marine communities are dynamic (Berdjeb et al., 2018; Henriques et al., 2006) arising from the temporally and spatially fluid aquatic gradients (Jakacki et al., 2017; Storesund et al., 2017). Despite this, the microbial communities within fjords of the southern hemisphere (particularly NZ) remain unexplored. NZ fjords are part of a temperate climate,

unlike those found in Chile, Antarctica and Iceland. The different climates, fauna and flora changes, as well as morphological differences, such as depth, could influence the drivers of microbial community composition in fjords.

In the present study we characterised the microbial (both eukaryotic and prokaryotic) community composition of six fjords in New Zealand, and linked the observed variance in diversity and community composition to key environmental variables (i.e. depth, salinity, horizontal location, oxygen, temperature) (Logue et al., 2015). We hypothesised that, similar to other fjord systems around the world, depth and salinity would be key determinants of microbial diversity and community composition (Sakami et al., 2016; Storesund et al., 2017; Walsh et al., 2016; Ying et al., 2009). We also hypothesised a close coupling between changes in eukaryotic and prokaryotic communities shift along fjords. To test this, we collected samples from six New Zealand fjords at multiple depths and locations, utilising amplicon sequencing of the small subunit ribosomal rRNA gene (both 16S and 18S) to determine changes in microbial communities.

2. Materials and methods

2.1. Sampling. Samples were collected in November 2015 throughout Fiordland National Park (45.60° S, 167.36° E) (Fig. 1), specifically Breaksea Sound (45.5860° S, 166.7567° E), Chalky Inlet (45.9922° S, 166.6024° E), Doubtful Sound (45.3260° S, 166.9911° E), Dusky Sound (45.7246° S, 166.5065° E), Long Sound (46.0001° S, 166.762415° E), and Wet Jacket Arm (45.6415° S, 166.8413° E).

A total of 44 samples were collected from Breaksea Sound (8), Chalky Inlet (10), Doubtful Sound (10), Dusky Sound (10), and Wet Jacket Arm (6). At each site duplicate (2) samples were collected from inner and outer regions of the fjord, and for each region sampling was performed at two depths (0 and 10 m), exceptions including Breaksea Sound's

outer 10 m, Chalky Inlet's inner 10 m, Doubtful Sound's inner surface, Dusky Sound's inner 10 m, and Wet Jacket Arm's inner surface regions where 1, 1, 4, 1, and 4 samples were taken respectively. For higher resolution sampling, duplicate samples were collected at Long Sound from a transect starting at an outer location (Fig. 1 b) and moving inwards with sampling occurring at 2.47, 3.16, 4.73, 5.59, 8.47, 10.67, and 14.3 km from the outermost sample at a depth of 0 and 10 m, exceptions including samples at 2.47, 3.16, 4.73, and 10.67 km at 10 m where no samples were taken, at 8.47 km where only a single sample was taken along the surface for prokaryotes, and at 10.67 km at the surface where only a single eukaryotic sample was taken. An additional depth profile was collected at 8.47 km from the outermost site with duplicate samples collected at 0, 10, 40, 100, 200, and 360 m depths (Fig. 1 b), exceptions including surface, 10 m, and 200 m samples were 4, 3, and 3 samples were taken respectively.

A CTD sensor system (SBE-25 0352) was used for vertical profiling of salinity (conductivity), temperature, and dissolved oxygen. Water samples were collected in 10 L Niskin bottles for subsequent analysis.

2.2. DNA extraction. A 0.5 to 1 litre subsample of water was filtered through a 0.22 µm (diameter of 47 mm) polycarbonate filter prior to freezing and storage at -20 °C during transport to the lab and finally stored at -80°C until further processing. Total community DNA was extracted from filters using the MoBio DNeasy® PowerSoil® Kit (MoBio, Solana Beach, CA, USA) in accordance to a modified manufacturer's protocol. Samples were bead beaten in a Geno/Grinder for 2 × 15s instead of vortexing at maximum speed for 10 minutes. All extracted DNA was stored at -20°C until further use.

2.3. Total community profiling using small subunit ribosomal rRNA gene (SSU rRNA) amplicon libraries. Community profiles were generated using barcoded 16S (targeting the V4 region: 515F (5′-NNNNNNNNGTGTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′)) or 18S 1391f (5'- GTACACACCGCCCGTC-3') and EukBr (5'- TGATCCTTCTGCAGGTTCACCTAC-3') rRNA gene primers as per the Earth Microbiome Project protocol(Caporaso et al., 2012). Barcoded samples were then loaded onto separate Illumina HiSeq (16S) or MiSeq (18S) 2 x 151 bp runs (Illumina, Inc., CA, USA).

Raw community profiles were analysed using the Quantitative Insights Into Molecular Ecology (QIIME) 1.9.1 open-reference operation taxonomic unit-picking workflow (Caporaso et al., 2010). Default parameters, including a read length >75 bp, minimum number of consecutive high quality base calls to include a read as a fraction of the input read length of 0.75, a Phred quality score of 3, no ambiguous bases, and no mismatches in the primer sequence (Bokulich et al., 2013) were used. Analysed sequences were all >136 bp in length. Raw sequences were demultiplexed with no ambiguous bases, using only forward reads and the split_libraries_fastq.py command. This has been shown to increase sequence depth per sample and analysis speed while producing comparable results to paired end data (Werner et al., 2012). Operational taxonomic units (OTUs) were generated by clustering at 97% for 16S and 99% for 18S similarity using UCLUST (Edgar, 2010) and an open reference strategy based on reference sequences located in the SILVA database (release 128) using the QIIME pick_open_reference_otus.py command (Quast et al., 2013). OTUs were classified to 7 taxa levels (kingdom, phylum, class, order, family, genus, OTU) using BLAST (Altschul et al., 1997) with a maximum e-value of 0.001 against the SILVA database.

The 16S sequence pool was subsampled and rarefied 10 times to a depth of 22,000 sequences to eliminate biases in sampling depth, while the 18S sequence pool utilised a depth of 6,600 sequences. The 10 resulting tables were merged into a single OTU table with a total sequence depth of 220,000 and 66,000 sequences per sample respectively. The data was exported as a biom (json) file for further processing.

2.4. Microbial community analysis. All data analysis was carried out within RStudio (R Core Team, 2018) version 1.1.453 (RStudio Team, 2016), and visualised using the ggplot2 package (version 3.0.0) (Wickham, 2016) unless otherwise stated. Sample counts were transformed using transform_sample_counts() in the phyloseq package (version 1.24.2) (McMurdie and Holmes, 2013) to account for multiple rarefactions. Individual OTU abundances were divided by 10 to provide a mean. To avoid counts represented by fractions all data was rounded to the nearest possibility (using the round() command from the stats package – version 3.5.1) prior to downstream analysis. The α-diversity measures observed richness and Shannon were calculated using the estimate_richness() command within the phyloseq package, while Pielou evenness was calculated using the evenness() commands from the microbiome package (version 1.2.1) (Lahti et al., 2017). Significance was calculated using a Kruskal-Wallis (KW) test using the kruskal.test() command, from the stats package. Interactions between geographical parameters were calculated using the interaction() command from the base package. Visualisation of β-diversity utilised plot_ordination() from the vegan package together with adjustments from the ggplot2 package (version 3.0.0). Significant correlations between environmental variables and β-diversity were calculated using the PCA() command from the FactoMineR package (version 1.4.1) (Lê et al., 2008). A mantel test was used, as previously described, to identify significant community changes in relation to depth, horizontal location, and the fjord of origin.

Significant microbial community genera changes were calculated using the EdgeR package (version 3.22.3) (Robinson et al., 2009) and an exact test across both the five-fjord and Long Sound fjords. All p values were FDR adjusted using Bonferroni via the p.adjust() command from the stats package. All significantly changing organisms were displayed at the phyla level for ease of visualisation.

Medians were calculated using the ddply() command from the plyr package (version 1.8.4) (Wickham, 2011). Standard error, standard deviation and the 95% confidence interval calculated using summarySE() from Rmisc (version 1.5) (Hope, 2013), and arrange() from the dplyr package (version 0.7.6) (Wickham, 2018).

2.5. Statistical analyses. Physicochemical parameters across and within Long Sound were visualised using the graphics package (version 3.5.1) within RStudio (R Core Team, 2018). Mantel tests (vegan package version 2.5-2) (Oksanen, 2008), were used to determine significantly changing physicochemical parameters across geographical (depth, horizontal location, and fjord of origin) parameters.

2.6. Data availability. The sequence data from this study have been deposited in NCBI under BioProject PRJNA540153. All data generated and/or analysed during the study is available within the GitHub repository, https://github.com/SvenTobias-Hunefeldt/Fiordland_2019/.

3. Results

3.1. Alpha diversity within fjords

OTU richness remained constant across the surface transect in Long Sound fjord for both prokaryotic (1050 ± 183) and eukaryotic (388 ± 44) communities (Spearman, p value

>0.05)(Fig. 2a-b). At 10 m depth, observed OTUs increased (Spearman: prokaryotes rho = 0.83, p value = 0.01; eukaryotes rho = 0.76, p value = 0.05) towards the head (landside/in) of the fjord resulting in a near doubling of richness (Fig. 2a-b) compared to the mouth of the fjord (seaside/out). On average, across the length of Long Sound, no significant differences (KW, $p > 0.05$) were detected in alpha diversity between surface and 10 m richness for either prokaryotes or eukaryotes.

A depth profile in the deepest sampled site of the same fjord (8.5 km) showed the prokaryotic richness increasing with depth, with a maximum observed (1503) at 100-200 m, whereas eukaryote's highest richness (599) was located at 10 m (Fig. 2c-d). Overall half as many eukaryotes were identified in the sample compared to prokaryotes (Fig. 2).

When richness patterns between outer and inner sites were compared across 5 fjords (Breaksea Sound, Chalky Inlet, Doubtful Sound, Dusky Sound, and Wet Jacket Arm), inner surface communities exhibited higher prokaryotic richness (Fig. 2e), although high variance was observed across fjords. Both depth (KW, X^2 of 19.55, p value < 0.01) and location within the fjord (KW, X^2 of 9.40, p value < 0.01) were significantly associated with changes in observed richness, with strong interactions between them (KW, X^2 of 29.75, p value < 0.01) (Table S1 and Fig. 2e).

3.2. Beta diversity within Long Sound

Long Sound's microbial community was dominated by a few key phyla, with Proteobacteria and Bacteroidetes being the most abundant prokaryotes, while SAR (Stramenopila, Alveolate, and Rhizaria) and Opisthokonta were the most abundant eukaryotes (Table S2-S4). Changes in communities (β-diversity) within the same fjord where strongly linked to depth (Fig. 3). At Long Sound, samples along the horizontal transect were primarily clustered based on depth (0 vs. 10 m), for both prokaryotes (adonis (ado), r2 = 0.24,

p value < 0.01, ANOSIM (ANO), R = 0.57, p value < 0.01) and eukaryotes (ado, r2 = 0.26, p value <0.01, ANO, R = 0.66, p value <0.01) (Fig. 3a-b), with interactions by location (Table S5). The same depth stratification was seen on the vertical axis of Long Sound, showing a clear difference between depths for eukaryotes (ado, r2 = 0.23, p value < 0.01, ANO, R = 1, p value <0.01), specifically between the surface, 10 m, and ≥40 m samples (Fig. 3c-d), but not prokaryotes (p value > 0.05). However, prokaryotic community changes were significantly associated with changes in salinity, oxygen and their interaction (Table S5), which were themselves associated with depth stratification (p value <0.05). For eukaryotic communities, stratification along the horizontal and vertical axis was correlated significantly with all tested environmental parameters excluding horizontal location (Table 14). Prokaryotic stratification based on depth was most correlated along the vertical NMDS axis (Fig. 3c-d), while the eukaryotic NMDS1 showed separation between communities above 10 and those below 40 m. The NMDS2 separated surface, 10 m, and below 40 m, the surface and 10 m communities shown to be more dissimilar than those below 40 m. Outer region prokaryotic and eukaryotic Long Sound samples clustered together, unlike the innermost samples (Fig. 3a-b). This pattern was also noted in the prokaryotic five-fjord NMDS (Fig. 4).

3.3. Significantly changing taxa

Salinity change patterns along the transect in deeper (10 m) samples are similar to the ones observed in surface samples, but less pronounced. Oxygen levels remained constant along the surface samples and showed a decrease towards the Fjord's head in 10 m samples (Fig. 5a-b). The shifts found in community composition seemed to correlate with these changes. For example, along the transect, shifts in dominant taxa in the surface samples seemed to correlate to sharp changes in salinity. The most notable shift in taxa occurred between 5.6 and 8.5 km for both prokaryotes and eukaryotes, where the Bacteroidetes and

Opisthokonta decreased from the outermost to innermost sample along a region with steep salinity changes (Fig. 5).

When taxonomic changes where explored using the depth profile in Long Sound, large rearrangements were observed at the domain and all eukaryotic phyla level organisms in accordance to mixing regions (Fig. 6). At the surface, Eukaryota (mostly Opisthokonta) clearly dominated the community (>75%), but as depth increased the relative abundance of eukaryotes decreased within the first 40 m to <60%, reaching stable levels at depths > 100 m (<38%, mostly SAR). Concerning prokaryotes, Archaea were almost absent at the surface (<5%), increasing with depth, and stabilizing at around 40 m (>50%). In contrast, Bacteria remained largely unaffected by depth. At phylum level, changes in taxa abundance were only pronounced for some groups. The relative abundance of SAR increased with depth, while Opisthokonta decreased. The prokaryotic phyla Proteobacteria and Bacteroidetes, and the eukaryotic phyla SAR and Opisthokonta remained the most abundant taxa within Long Sound that significantly correlated with most tested environmental variables (Fig. 6, 7). Shifts in abundance for different groups where consistent within domains, but differed across them. Changes in prokaryotic taxa abundance occurred at 100-200 m, whereas eukaryotic shifts occurred much closer to the surface at 40 m.

While some prokaryotes (i.e. Proteobacteria, Bacteroidetes, and Cyanobacteria), showed significant correlations with all tested environmental variables, this was not the case for eukaryotes (Fig. 5, 7). No eukaryotic taxa was found to significantly correlate with oxygen or temperature. However, we did see an inverse relationship for all Long Sound's community patterns, most commonly between Proteobacteria and Bacteroidetes, and SAR and Opisthokonta (Fig. 5, 7).

4. Discussion

Our analyses indicate that a correlation exists between the diversity and community structure, and the depth and salinity conditions within six New Zealand fjords. These results are consistent with previous studies suggesting that changes in a fjord's microbial diversity are primarily correlated with environmental conditions associated with depth, such as salinity (Bordalo and Vieira, 2005; Crump et al., 2004; Herlemann et al., 2011), light (Hölker et al., n.d.), organic matter (POC (Moncada et al., 2019), PON (Moncada et al., 2019), DOC (Li et al., 2012)), tannins (Baptist et al., 2008) and temperature (Berner et al., 2018; Hobbie, 1988). A LSL (low salinity layer), distinct from the lower marine layer (Citterio et al., 2017), is common across a fjord's surface. Physicochemical differences between the marine and LSL layers could be responsible for large diversity and taxa shifts within the upper 10 m (Citterio et al., 2017). Hence, salinity could play a role for both the vertical and horizontal distribution of the community or act as a proxy for LSL mixing. Mixing zones, known to be particularly diverse due to hosting organisms from both environments (Gibbons and Gilbert, 2015), presented a particular strong response correlated with salinity changes (Cloern et al., 2017). The observed shift in salinity could most likely be associated with Fiordland's characteristic extremely high rainfall levels and thus large freshwater inputs (NIWA, 2016) resulting in large differences in environmental conditions between the surface and the 10 m depth strata. Shifts in salinity, combined with high tannin concentrations, which limit light penetration to approximately 50 m (Nelson et al., 2002), could influence a rapid change in diversity patterns and taxa between depths, especially in the first 10 m. In addition, the retention times of different water masses in the fjords differ enormously. While the surface water stays several hours in the fjord and flows seawards constantly, the seawater below is slowly entrained staying several months in the fjord (Goebel et al., 2005) depending on freshwater inputs. The relatively shallow nature of the terminal sills at the fjord entrances (30 to 150 m (Stanton and

290 Pickard, 1981)) limits the depth-associated effects by limiting the maximum salinity of the
291 marine water within the fjord, since only the relatively shallow (and thus less saline) marine
292 water enters and flows up the fjord (Pickrill, 1993; Stigebrandt, 2012; Talley et al., 2011),
293 resulting in a less saline mixing layer between the marine water and LSL that would be
294 associated with oceanic deep water. The relationship between salinity and depth
295 (Maciejewska and Pempkowiak, 2014), is sustained on the scale of 100's of meters within the
296 ocean (Cummins, 1991) rather than the smaller scale relationship found within lakes (Garcia
297 et al., 2013). But overall, Fiordland contains more unique characteristics that increase the
298 effect of depth rather than those that decrease depth's effect on its community.
299
300 **4.1. Alpha diversity patterns were driven by depth and salinity**
301 Mixing due to currents, wind or semi-continuous external freshwater inputs (e.g. waterfalls
302 along the fjord walls) are likely responsible for the constant microbial (both prokaryotes and
303 eukaryotes) richness observed along Long Sound's surface as well as the varying retention
304 times between the surface and 10 m layer. Microbial richness at 10 m depth decreased
305 towards the fjord's mouth, possibly caused by the lower levels of mixing and higher water
306 retention times within Long Sound. Oxygen concentration decreases at 10 m towards the
307 fjord's head. Oxygen being consumed during the heterotrophic respiration of organic matter
308 and a lack of renewal from atmospheric exchange due to the LSL, which persistently
309 overcaps the underlying seawater.
310 The microbial diversity pattern found at the surface does not display a clear
311 progression related to horizontal location, while at 10 m a seeming region-like richness and
312 taxa change is noted for both prokaryotes and eukaryotes (Fig. 2, 3), most likely due to the
313 varying retention time (Goebel et al., 2005) and dilution effects. The relative longer retention
314 time at 10 m (compared to the LSL) and less wind mixing could allow for the establishment

315 of more complex communities, a community increasing in complexity and diversity as it ages
316 (Donlan, 2002). While the surface exhibits similar richness levels across the horizontal axis,
317 its high flow rate corresponding to a high rate of horizontal dispersal (Campbell and
318 Kirchman, 2013), and the homogeneity of phytoplankton communities within the LSL (and
319 associated heterotrophic community) (Goebel et al., 2005) prevent the establishment of
320 strong horizontal structure in microbial communities.

321 Observed richness along the vertical Long Sound axis correlated significantly with
322 salinity but not depth on microbial diversity, corroborating the strong influence of the surface
323 freshwater layer, constantly fed by external freshwater sources. Neither temperature nor
324 oxygen showed a correlation with richness patterns, even though these variables are
325 commonly considered to be very closely associated with depth. Much like marine snow often
326 correlates with depth. Marine snow comes from the sinking of surface organic matter and
327 organisms towards greater depths (Mestre et al., 2018), as such it is possible for microbes
328 growing at the surface to be found at unexpected depths in an environment unsuitable for
329 their growth. Much like marine snow, the dispersal effect, based upon the fact that due to
330 their small size microbes disperse over large distances (Foissner, 2006), allows for microbes
331 to be found in environments unsuitable for their optimal growth.

332 When taxonomic richness is compared among the six fjords, Doubtful Sound had a
333 much higher richness level within the surface layer of the inner fjord. This 40 km long fjord
334 is characterised by a larger LSL produced by the Manapouri Power Station freshwater output
335 (Gibbs et al., 2000; Nelson et al., 2002). The deeper and more persistent LSL could have
336 resulted in the observed increased microbial richness. An elevated flow rate and strong
337 separation between the LSL and the underlying seawater layer likely prevented migration of
338 microbial communities to the 10 m layer at the fjord's head, also potentially decreasing
339 vertical fluxes associated with marine snow. The inner surface region of five of the fjords

studied presented higher microbial taxonomic richness levels, in contrast with the trend observed in Long Sound. As such, the patterns observed among the six fjords demonstrated that while microbial richness patterns within these fjords were correlated with depth, it was not in accordance to our initial hypothesis. Overall, our results indicate that both prokaryotic and eukaryotic richness patterns within all tested fjords present a clear correlation with depth.

4.2. Depth effects drove community changes between sites

Long Sound's prokaryotic and eukaryotic outermost communities presented more similarities amongst themselves than the innermost communities, possibly due to a smaller LSL in those outer regions and the effects of increased mixing on horizontal dispersal. The innermost communities at different depths were just as dissimilar to each other as they were to the outermost communities, indicating a clear effect of strong vertical gradients in environmental conditions linked to the presence of a deep and distinct LSL. The LSL is at its deepest at the fjord's head where the main freshwater runoff is located, and in Long Sound because of topographic isolation of the inner fjord basin the LSL is thick and has a higher retention time than in the other fjords. Towards the outermost regions, across the narrow passage that connects Long Sound with the outer coast Preservation Inlet, the LSL becomes much shallower, at times disappearing (Wing, 2009) and is subject to advection and mixing by ocean waves. As such, the outermost communities at both the surface and 10 m exist in non-LSL-influenced marine water. This contrasts with the communities closer to the fjord's head, where the surface community is located primarily within the LSL, while the community at 10 m is found within LSL-influenced marine water. This degree of marine influence was also seen on the prokaryotic and eukaryotic vertical Long Sound axis NMDS plots. Grouping of communities into three separate layers was found, possibly due to depth associated environmental condition changes, specifically salinity and oxygen. However, prokaryotic

communities of different depths were less dissimilar compared to eukaryotic communities. Both prokaryotes and eukaryotes showed that depth differences, and thus degree of marine influence, resulted in different communities. Based on this we hypothesised that each fjord would contain its own unique microbial community associated with the distinct environments within each fjord (e.g. depth, LSL depth, freshwater input, fjord length, entrance depth, etc.). Non-significant fjord clustering was noted for prokaryotes, the inner surface samples all clustering to the left of other samples from the same fjord, possibly due to the freshwater input carrying soil organisms from the fjord's head. The separation of the inner surface samples reflects the increase in richness of the five-fjords but not the Long Sound richness patterns found in our study. Interestingly, the amount of freshwater appeared to influence the five-fjord NMDS, since Chalky Inlet (containing the smallest LSL) and Doubtful Sound (with the largest LSL) were located at opposite sides of the NMDS plot. Overall, diversity patterns matched with depth associated environmental changes across Long Sound. However, those same trends were not visible within the five-fjord diversity and community patterns.

4.3. Fiordland fjords community composition changes are mainly due to the effects depth has on environmental variables.

Our study was the first to simultaneously investigate patterns in both prokaryotic and eukaryotic community diversity within fjords. To date, very few studies have focused on diversity patterns in fjordic environments, particularly those of the eukaryotic community, often excluding large taxa and targeting specific groups such as protists (Orsi et al., 2012; Piquet et al., 2010). The results obtained on dominantly identified phyla in the present study are consistent with previous reports on marine and fjord systems, which were dominated by Proteobacteria (Aldunate et al., 2018; Sinha et al., 2017; Spietz et al., 2015; Vander Roost et al., 2018; Zaikova et al., 2010), Bacteroidetes (Aldunate et al., 2018; Fernández-Gómez et al.,

2013; Sinha et al., 2017; Spietz et al., 2015), Opisthokonta (Del Campo et al., 2015), and SAR (Guillou et al., 2008).

Proteobacteria include many different phylogenetic groups capable of diverse functional ability, from consuming dissolved organic matter (Wagner et al., 2006) to Sulphur oxidation (Frigaard et al., 2006; Yamamoto and Takai, 2011). Marine associated Bacteroidetes degrade particulate organic matter, containing significantly more proteases than non-marine Bacteroidetes (Fernández-Gómez et al., 2013). Marine associated Bacteroidetes attachment to phytoplankton may thus influence Bacteroidetes distribution, as has been previously observed within blooms (Pinhassi et al., 2004; Smith et al., 2017; Teeling et al., 2012). Cyanobacteria, a photoautotrophic oxygen producing prokaryote phyla (Hamilton et al., 2016), showed high abundance at the surface but was mostly absent at greater depths. The observed pattern is consistent with those found in other marine systems, abundance decreasing with light penetration and availability (Cantonati et al., 2014).

Opisthokonta are a diverse group of eukaryotes including organisms from both the animal and fungal kingdoms (Adl et al., 2018; Shalchian-Tabrizi et al., 2008), making it hard to determine the role they play within marine systems. Key characteristics of Opisthokonta include synthesis of extracellular chitin, cyst/spore/cell walls of filamentous growth, hyphae, and the extracellular digestion of substrates (Adl et al., 2018). Only 5% of SAR are from well sampled families making them a poorly understood phyla (Grattepanche et al., 2018). SAR is made up of Stramenopila, Alveolate, and Rhizaria. Stramenopila are mostly made up of autotrophic primary CO_2 producers. A shared ecological function has not yet been determined for Alveolate, which made up the majority of the SAR distribution patterns (Table S2-S4). Alveolata are characterised by the presence of cortical alveoli (membrane-bound sacs underlying the cell membrane). Rhizaria is made up of a diverse range of free-living heterotroph lineages. Photosynthetic Rhizaria have also undergone photosynthetic

415 endosymbiosis. But difficulty in isolation and cultivation makes Rhizaria and other SAR
416 clades hard to study within laboratory settings.

417 Prokaryotes and eukaryotes shifted at different depths along vertical axis of Long
418 Sound. While the prokaryotic community shifted at 100 m depth, the eukaryotic community
419 shifted much closer to the surface at 40 m depth. The late shift of the prokaryotic community
420 is most likely due to the presence of a permanent sulphur mat at ~150 m (James et al., 2018),
421 while the eukaryotic community shift could be explained by the effects of light penetration,
422 due to the high tannin concentration in the water due to the land runoff in periods of high
423 rainfall. As such, even changes across the horizontal axis can be attributed to the differences
424 in environmental factors derived from the effects of depth.

425 The environmental factors of depth, horizontal location, and salinity consistently
426 correlated with both prokaryotic and eukaryotic diversity patterns. Oxygen and temperature
427 did not match the taxa distribution pattern as closely as depth, horizontal location, and
428 salinity. Thus, we hypothesise that oxygen and temperature were not as important as the other
429 environmental variables in determining taxonomic diversity patterns . Indeed, depth and
430 salinity, which always significantly correlated with community diversity, are likely much
431 more influential in determining microbial community assemblages established within a fjord.
432

433 **5. Conclusions**

434 For the first time depth and salinity have been shown to act as major factors on both
435 prokaryotic and eukaryotic diversity, community composition, and taxa patterns across and
436 within fjords. However, our exploratory study did not focus on individual organisms or their
437 interactions within the ecosystem. We instead focused on high taxa rank distributions due to
438 environmental parameters; prompted by the lack of similar studies. Subsequent studies
439 should focus on functional analyses across depths and individual interactions across the

ecosystem. A high-resolution sampling of all fjords should be utilised instead of a region-based analysis. Regional analyses allow the identification of general trends along a fjord but lack the power to assess individual fjords. Unique fjord characteristics (e.g. additional freshwater sources) make direct cross-fjord studies difficult; cross-fjord studies should instead assess conserved trends across multiple fjords. Further refining our understanding of microbial communities within a fjord and the ecological reasoning behind their distribution.

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Conflict of interest

The authors declare that they have no conflict of interest.

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735 **Figures legends:**

736 **Figure 1. Geographical location of sampling sites.** Geographical locations of sampling sites
737 in New Zealand, Fiordland (a) (Kahle and Wickham, 2013), and Long Sound's sampling
738 scheme (b).

739

740 **Figure 2. Alpha-diversity of Fiordland fjords.** Richness for both 16S (left side) and 18S
741 (right side) across Long Sound's horizontal axis (a, b), Long Sound's vertical axis (c, d), and
742 richness based on 16S for all five sampled fjords samples (e). OTU clustering as carried out
743 at 97% similarity.

744

745 **Figure 3. Microbial beta-diversity within Long Sound.** Beta-diversity based on 16S (left
746 panels) and 18S (right panels) data for Long Sound's horizontal axis (a, b) and its vertical
747 axis (c, d). Dissimilarity was assessed using Bray-Kurtis distance matrices based on OTUs at
748 97% similarity.

749

750 **Figure 4. Microbial beta-diversity of five fjords.** The fjord of origin, sample region, and
751 depth were used to identify sample origin. Dissimilarity was assessed using Bray-Kurtis
752 distance matrices based on OTUs at 97% similarity.

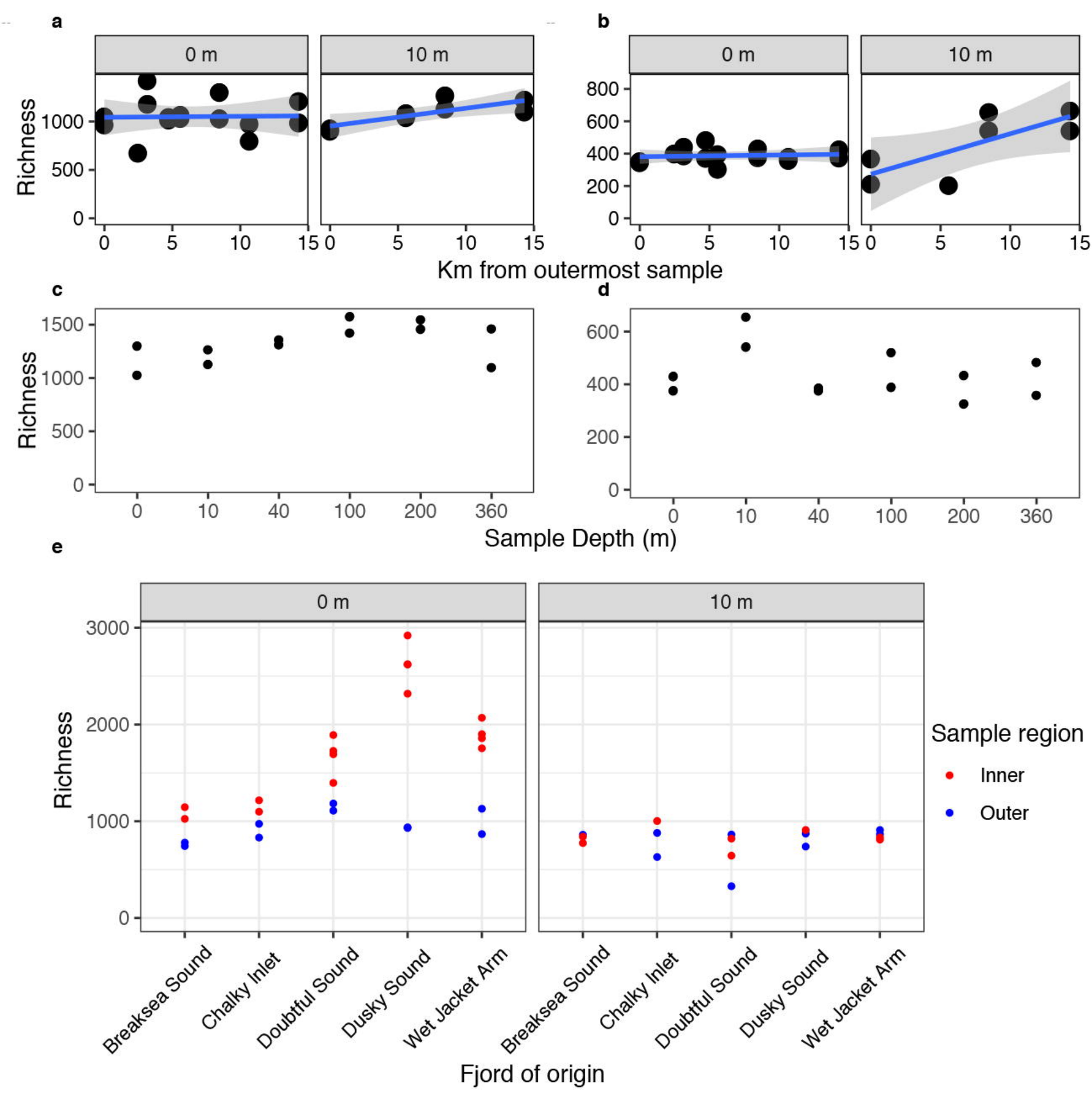
753

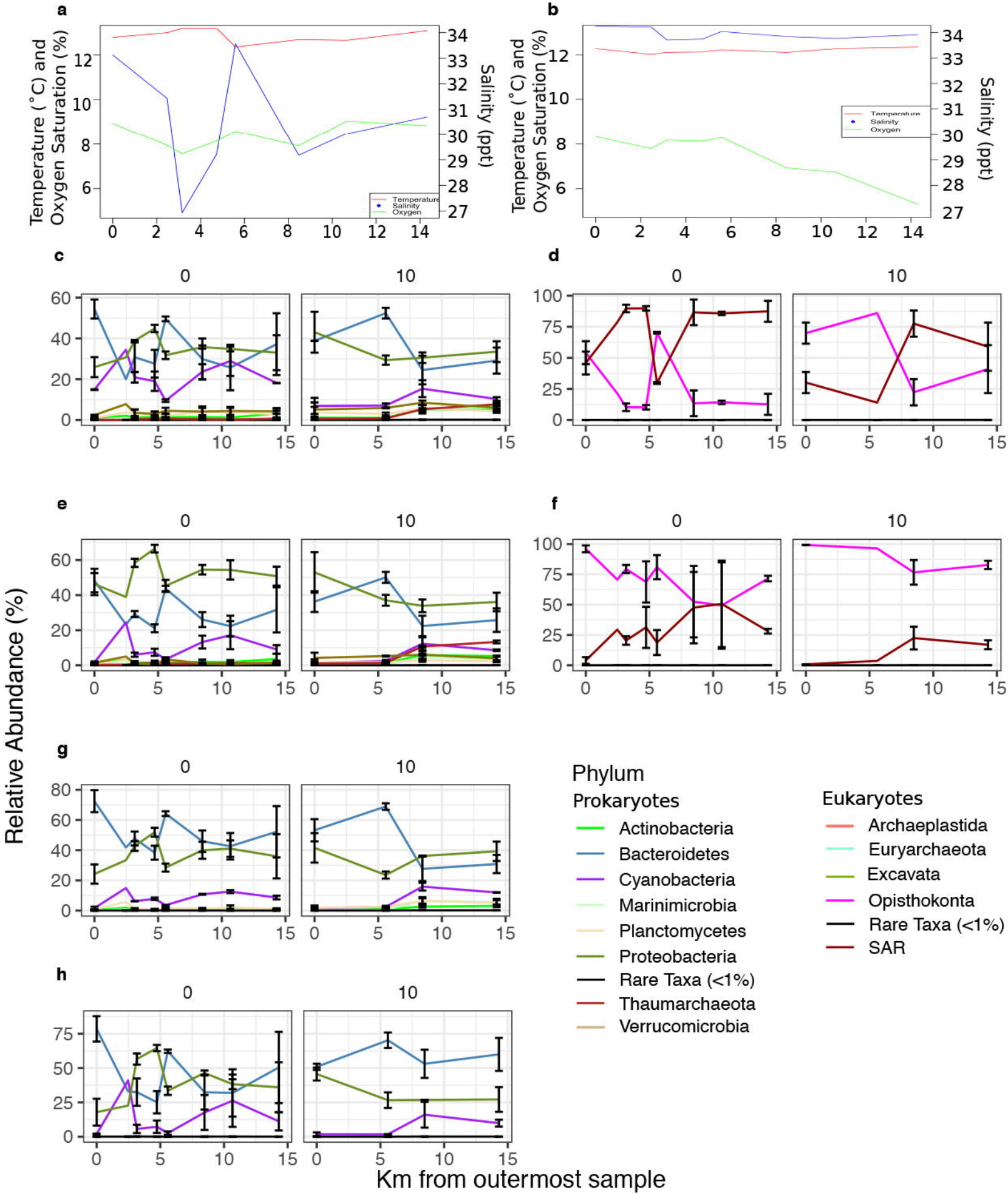
754

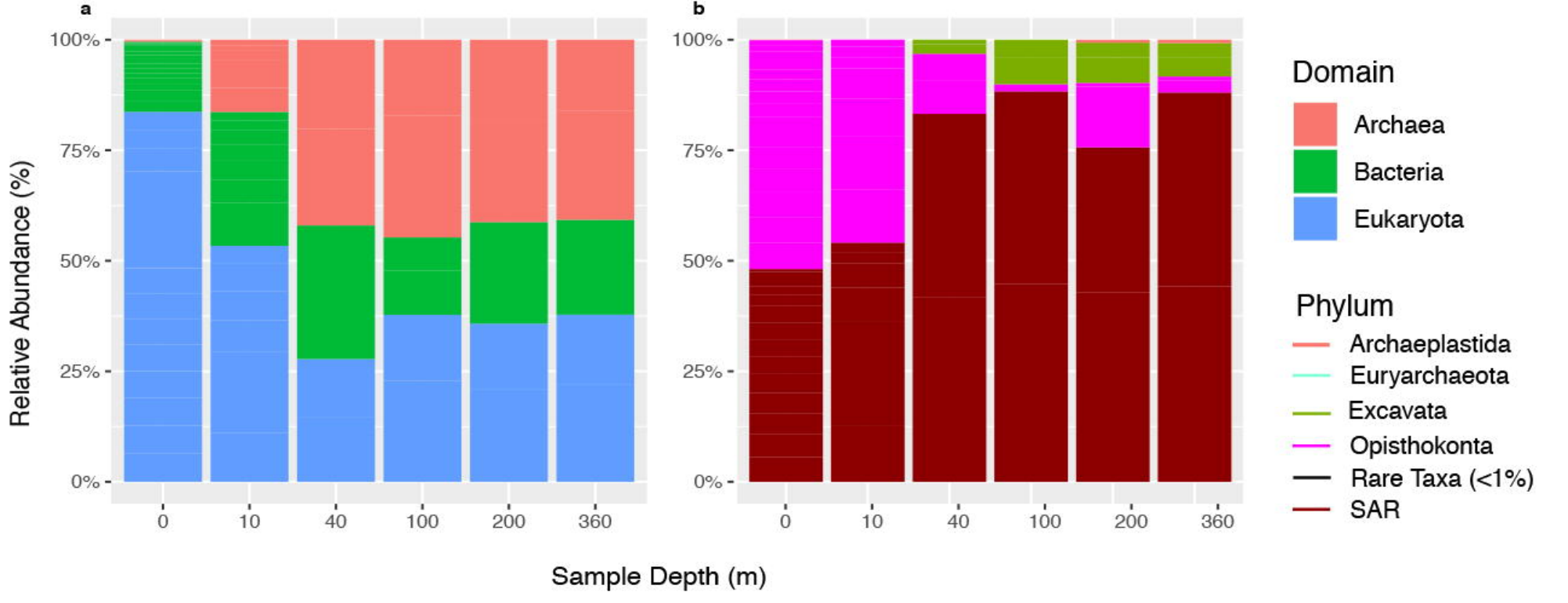
755 **Figure 5. Changes in environmental parameters and significantly associated taxa along**
756 **Long Sound's horizontal axis.** Environmental parameter changes were noted across Long
757 Sound's horizontal axis surface (a) and 10 m (b) in a discrete manner. Also shown is the
758 mean relative abundance of phyla across Long Sound's horizontal axis (c-h). Communities

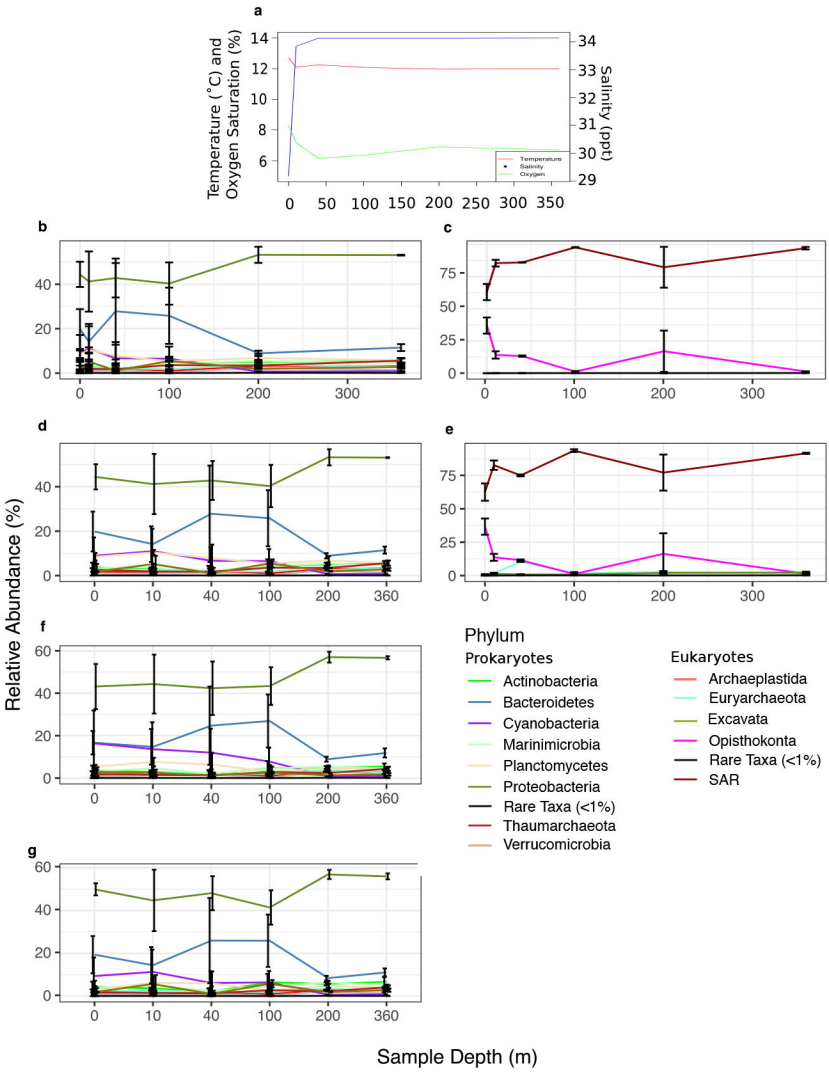
759 were based on 16S (left side) and 18S (right side) sequencing. The microbial community was
760 correlated against distance from the outermost sample (c, d), salinity (e, f), temperature (g),
761 and oxygen (h). Phyla <1% mean relative abundance have been identified and grouped into
762 Rare Taxa (<1%). Error bars represent standard error of the mean abundance from repeated
763 samplings. Mean relative abundance was calculated from pooled significantly correlated taxa.
764
765 **Figure 6. 18S sequencing based microbial community of Long Sound's vertical axis.**
766 Long Sound's vertical community is shown at both the domain (a) and phyla (b) level as
767 depth increases. Phyla <1% relative abundance have been identified and grouped into Rare
768 Taxa (<1%). Mean relative abundance was calculated from pooled taxa.
769
770 **Figure 7. Changes in environmental parameters and significantly associated taxa along**
771 **Long Sound's vertical axis.** Environmental parameter changes were noted across Long
772 Sound's vertical axis (a) in a discrete manner. Also shown is the mean relative abundance of
773 phyla across Long Sound's vertical axis. Communities were based on 16S (left side) and 18S
774 (right side) sequencing. The microbial community was correlated against distance from the
775 outermost sample (b, c), salinity (d, e), temperature (f), and oxygen (g. Phyla <1% mean
776 relative abundance have been identified and grouped into Rare Taxa (<1%). Error bars
777 represent standard error of the mean abundance from repeated samplings. Mean relative
778 abundance was calculated from pooled significantly correlated taxa.
779
780 **Table S1.** Kruskal-Wallis tests on observed richness in relation to depth, distance, fjord of
781 origin, salinity, oxygen, temperature for all studied fjords.
782

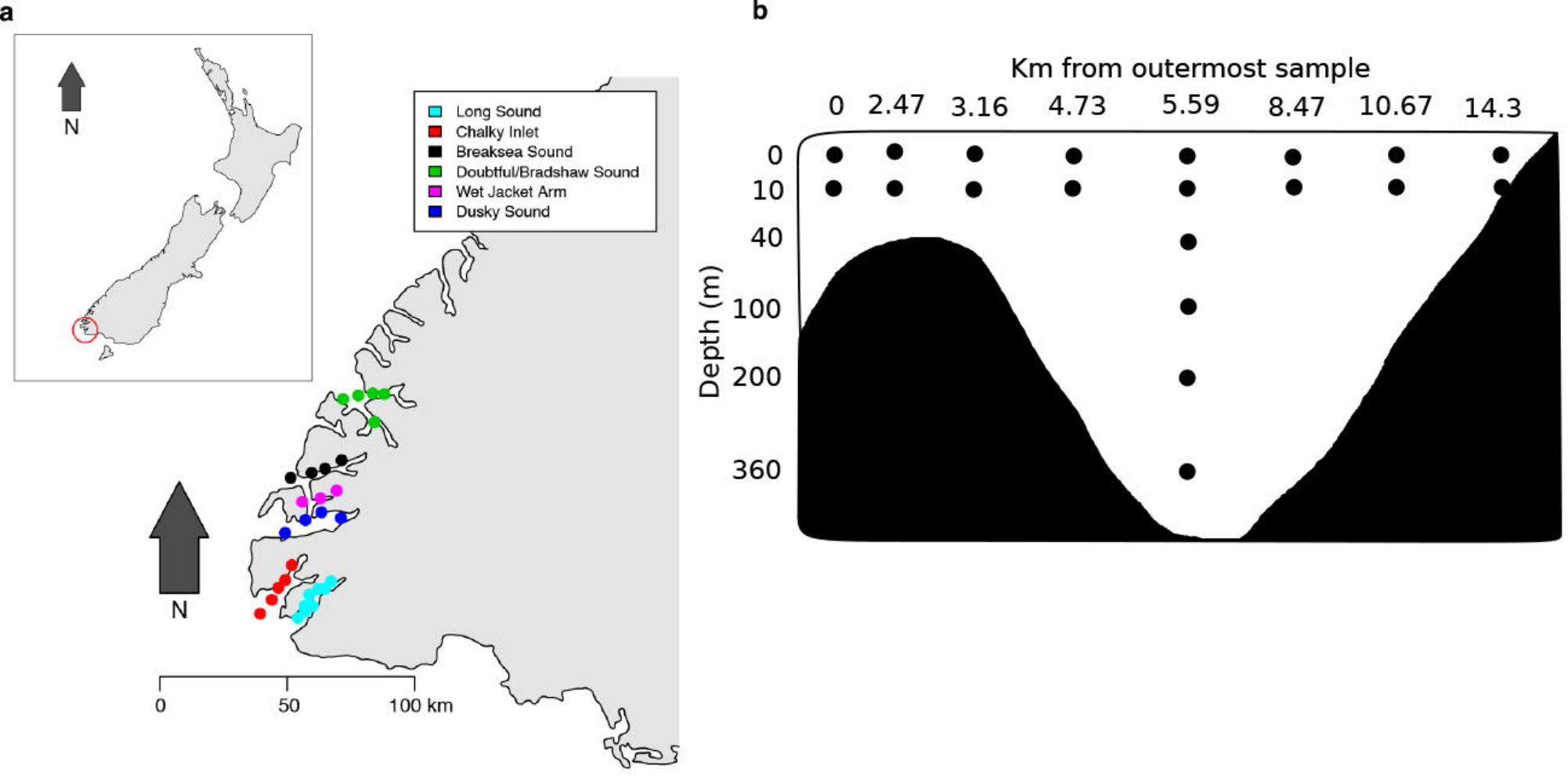
783 **Table S2.** Prokaryotic taxa significantly correlated with distance, depth, salinity, oxygen, and
784 temperature along the horizontal axis of Long Sound fjord.
785
786 **Table S3.** Prokaryotic taxa significantly correlated with distance, depth, salinity, oxygen, and
787 temperature along the vertical axis of Long Sound fjord.
788
789 **Table S4.** Eukaryotic taxa significantly correlated with distance, depth, salinity, oxygen, and
790 temperature along the horizontal and vertical axis of Long Sound fjord.
791
792 **Table S5.** Mantel tests on fjord taxa in relation to depth, distance, fjord of origin, salinity,
793 oxygen, temperature for all studied fjords.

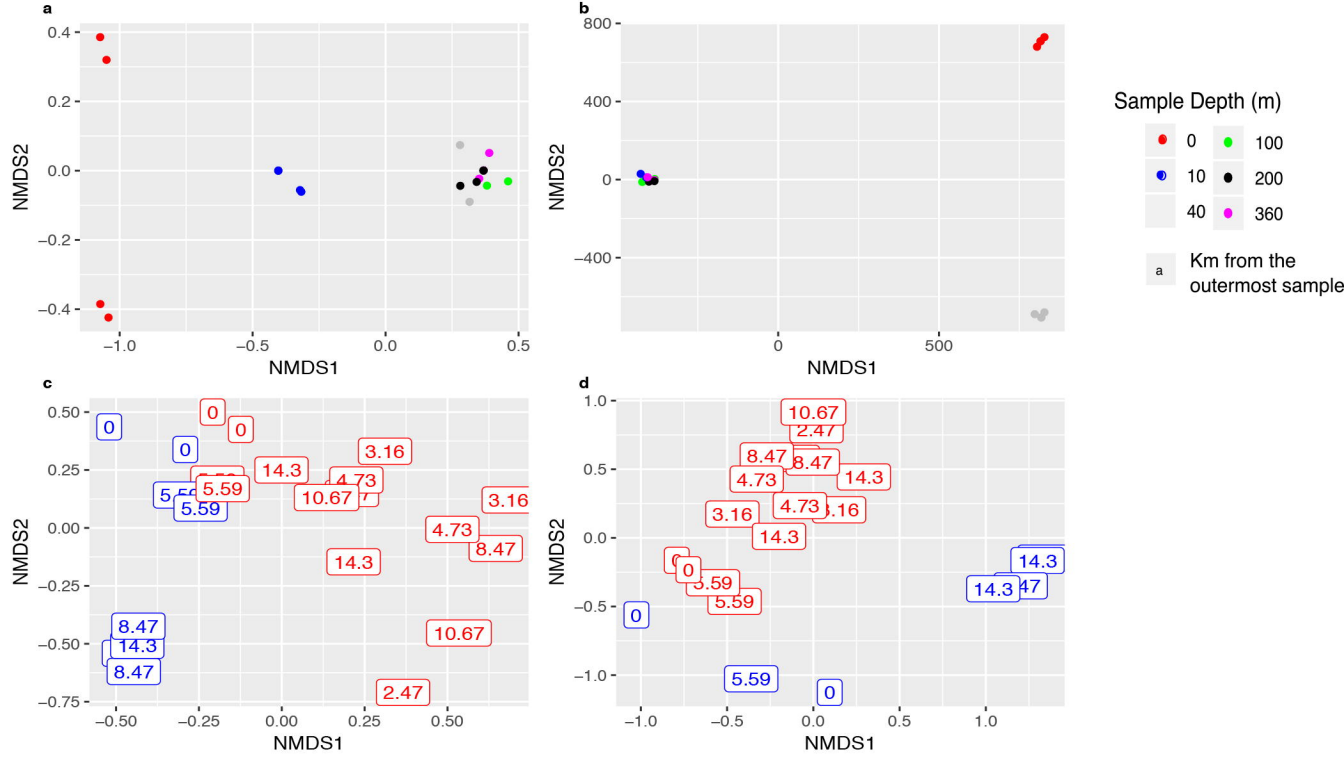

a
b
0 m
10 m
Richness
Km from outermost sample
c
d
Sample Depth (m)
e
Sample region
Inner
Outer
Breaksea Sound
Chalky Inlet
Doubtful Sound
Dusky Sound
Wet Jacket Arm
Fjord of origin

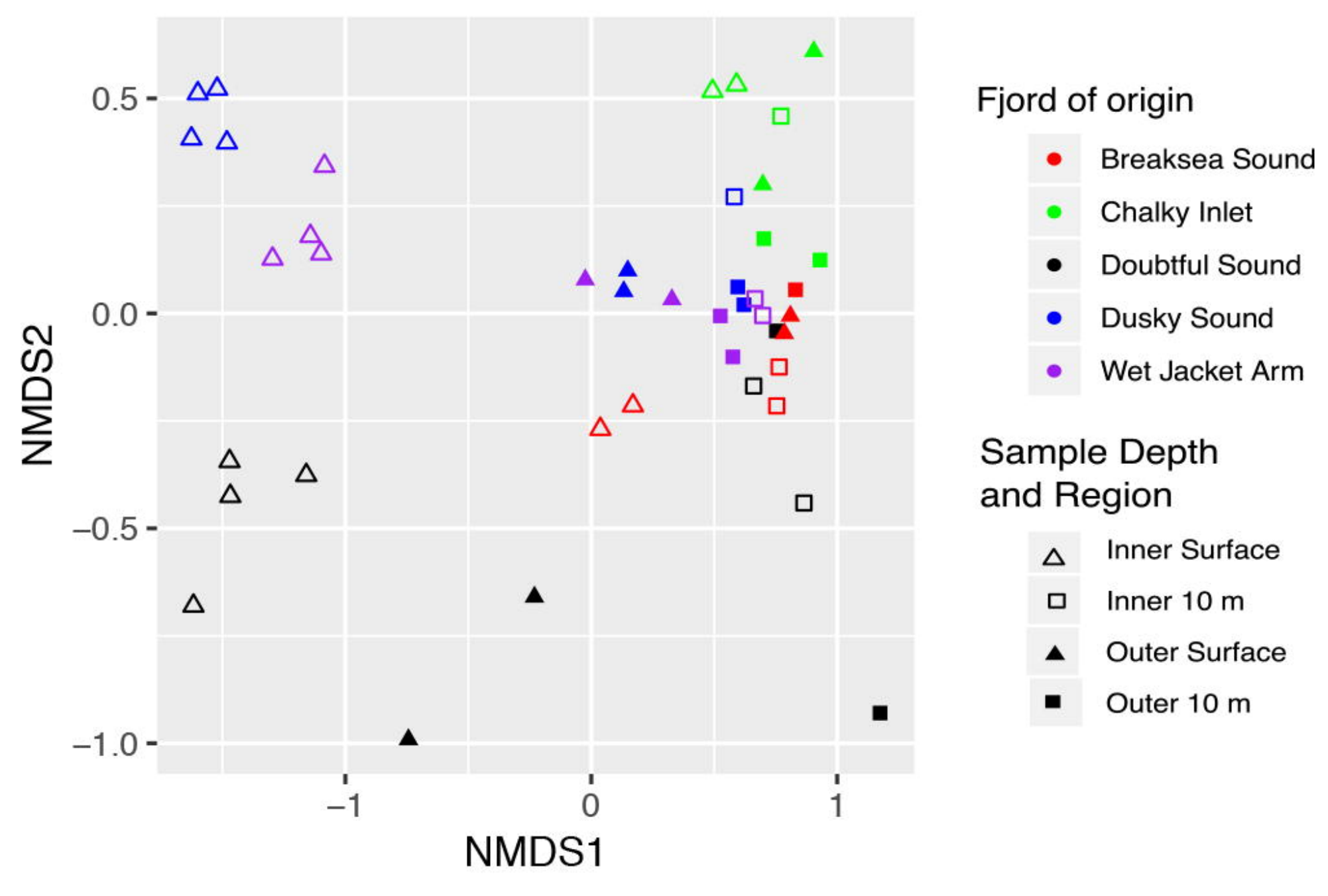

a
b
Temperature (°C) and Oxygen Saturation (%)
Salinity (ppt)
Temperature
Salinity
Oxygen
c
d
e
f
g
h
0
10
Relative Abundance (%)
Km from outermost sample
Phylum
Prokaryotes
Actinobacteria
Bacteroidetes
Cyanobacteria
Marinimicrobia
Planctomycetes
Proteobacteria
Rare Taxa (<1%)
Thaumarchaeota
Verrucomicrobia
Eukaryotes
Archaeplastida
Euryarchaeota
Excavata
Opisthokonta
Rare Taxa (<1%)
SAR


a
b
100%
75%
50%
25%
0%
Relative Abundance (%)
0
10
40
100
200
360
Sample Depth (m)
Domain
Archaea
Bacteria
Eukaryota
Phylum
Archaeplastida
Euryarchaeota
Excavata
Opisthokonta
Rare Taxa (<1%)
SAR


a
Temperature (°C) and Oxygen Saturation (%)
Salinity (ppt)
Temperature
Salinity
Oxygen
b
c
d
e
f
g
Relative Abundance (%)
Sample Depth (m)
Phylum
Prokaryotes
Actinobacteria
Bacteroidetes
Cyanobacteria
Marinimicrobia
Planctomycetes
Proteobacteria
Rare Taxa (<1%)
Thaumarchaeota
Verrucomicrobia
Eukaryotes
Archaeplastida
Euryarchaeota
Excavata
Opisthokonta
Rare Taxa (<1%)
SAR


a
N
Long Sound
Chalky Inlet
Breaksea Sound
Doubtful/Bradshaw Sound
Wet Jacket Arm
Dusky Sound
N
0
50
100 km
b
Km from outermost sample
0
2.47
3.16
4.73
5.59
8.47
10.67
14.3
Depth (m)
0
10
40
100
200
360


a
b
c
d
NMDS1
NMDS2
Sample Depth (m)
0
10
40
100
200
360
a
Km from the outermost sample


0.5
0.0
−0.5
−1.0
NMDS2
−1
0
1
NMDS1
Fjord of origin
Breaksea Sound
Chalky Inlet
Doubtful Sound
Dusky Sound
Wet Jacket Arm
Sample Depth and Region
Inner Surface
Inner 10 m
Outer Surface
Outer 10 m