Changes in Microbial Community Phylogeny and Metabolic Activity Along the Water Column Uncouple at Near Sediment Aphotic Layers in Fjords

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Abstract

Fjords are semi-enclosed marine systems with unique physical conditions that influence microbial community composition and structure. Pronounced organic matter and physical condition gradients within fjords provide a natural laboratory for the study of changes in microbial phylogeny and metabolic potential in response to environmental conditions. Photosynthetic production in euphotic zones sustains deeper aphotic microbial activity via organic matter sinking, augmented by large terrestrial inputs. We profiled microbial functional potential (Biolog Ecoplates), bacterial abundance, heterotrophic production (^{3}H-Leucine incorporation), and prokaryotic/eukaryotic community composition (16S and 18S rRNA amplicon gene sequencing) to link metabolic potential, activity, and community composition to known community drivers. Similar factors shaped metabolic potential, activity and community (prokaryotic and eukaryotic) composition across surface/near surface sites. However, increased metabolic diversity at near bottom (aphotic) sites reflected an organic matter influence from sediments. Photosynthetically produced particulate organic matter shaped the upper water column community composition and metabolic potential. In contrast, microbial activity at deeper aphotic waters were strongly influenced by other organic matter imput than sinking marine snow (e.g. sediment resuspension of benthic organic matter, remineralisation of terrestrially derived organic matter, etc.), severing the link between phylogeny and metabolic potential. Taken together, different organic matter sources shape microbial activity, but not community composition, in New Zealand fjords.

Introduction

Fjords are unique environments, representing modified marine ecosystems mixing freshwater, terrestrial and marine inputs. Influences on microbial community structure and function are linked to changes in environmental condition, including alternate organic carbon sources (e.g. tannins, terrestrial, marine and freshwater sources), salinity, nutrient, and light [1–3]. Moreover, due to these strong environmental gradients, fjords are ideal natural laboratories to study marine microbial communities and phylogenetic and functional diversity controls due to strong environmental gradients. However, the energy sources supporting primary production and heterotrophic activity in fjords, and how they change in relation to observed community changes, remain poorly defined. In open ocean systems primary productivity by surface phytoplankton mediates the downward flux of particulate carbon, transferring energy to aphotic zones. This unidirectional transfer of organic matter from surface to deeper layers is termed the biological carbon pump [4–6]. The process is expected to dominate in fjords where carbon inputs are predominantly linked to phytoplankton and chemoautotroph production [7–9], sustaining a significant portion of heterotrophic respiration [10]. Nevertheless, studies of benthic communities in fjords have demonstrated that microbial reworking of refractory organic matter from terrestrial sources is in some fjords a dominant source of carbon to deep communities [11–14]. Despite this, we lack an integrated view of microbial metabolic potential within fjords and specific information about the composition of microbial populations and how they are linked to the available range of organic matter sources. Resolution of these associations will provide the basis for a mechanistic understanding of how organic

matter is processed in fjords and increased understanding of how this ecosystem is sustained and shaped.

In a recent study we examined for the first time the patterns in microbial community composition relative to variability in environmental factors among fjords in the New Zealand Fiordland system [15]. Nevertheless, links between patterns in phylogenetic and functional diversity in these fjords remained unresolved. In the present study we utilised profiling of the functional potential of microbes (via Biolog Ecoplates), bacterial abundance, heterotrophic production (via ^{3}H-leucine incorporation) and prokaryotic/eukaryotic community composition (via 16S and 18S rRNA amplicon gene sequencing) to compare community metabolic diversity and potential, and how it related to known drivers of microbial community changes across six different fjords in New Zealand. We aimed to reveal how metabolic potential changed along environmental gradients within fjords and how these patterns are linked to community composition changes. We hypothesised that microbial community function and composition were linked, and both would decrease with depth due to decreased abundance of photosynthetically produced organic matter.

Methods

Sample collection, DNA extraction, and sequencing

Samples were collected, DNA extracted and sequenced and community profiles generate according to the protocol outlined in Tobias-Hünefeldt et al 2019 [15]. All sequence data from this study has been deposited in NCBI under BioProject PRJNA540153.

All data analysis was carried out using R version 3.6.1 within RStudio [16], and visualised using the ggplot2 package (version 3.2.1) [17] unless otherwise stated. All code and associated files are available at https://github.com/SvenTobias-Hunefeldt/ Fiordland_2021/.

Carbon utilisation profiling, and bacterial abundance and productivity

Carbon utilisation profiles were determined using Biolog EcoPlatesTM loaded with water collected in 10 L Niskin bottles attached to the CTD rosette. 150 µL of sample were utilised per Biolog Ecoplate well, and incubated for 7 days at 4˚C, colour patterns were assessed at OD A590 nm.

Samples incubated in the dark for 10 minutes and then preserved with glutaraldehyde (final concentration of 2%) and frozen with liquid nitrogen prior to counting. Thawed cells were stained with a final concentration of 10µL ml^{-1} SYBR green I fluorescent staining for 10 minutes., samples underwent bacterial abundance quantification using the FACS Canto II flow cytometer (Benton & Dickinson, USA) following methods in Gasol and Del Giorgio, 2000 [18].

Leucine incorporation assays were also used to quantify heterotrophic bacterial productivity, following the centrifugation protocol outlined in Smith and Azam, 1992 [19]. Triplicate 1.2mL samples received a saturating concentration (40 nmol l − 1) of 3H-Leucine (Perkin–Elmer, specific activity = 169 Ci mmol − 1). The addition of 120 µl of 50% trichloroacetic acid (TCA) 10 min prior to isotope addition established controls. Microcentrifuge tubes were incubated in the dark at in situ temperature for 1 h. Leucine incorporation in triplicate samples was stopped with the addition of 120 µL ice-cold 50% TCA. Subsamples and controls were kept at − 20°C until centrifugation (at ca. 12,000g) for 20 min, followed by aspiration. Finally, 1 ml of scintillation cocktail was added to the microcentrifuge tubes before determining the incorporated radioactivity after 24–48 h on a Tri-Carb® Liquid Scintillation Counters scintillation counter (Perkin–Elmer) with quenching correction.

Statistical analyses

Stats package generated PCA plots assessed Beta-diversity, with ggplot2, ggpubr, and ggbiplot package adjustments [17,20,21]. Phyloseq package generated NMDS plots corroborated findings. Dissimilarity utilised the vegan package vegdist() function and Bray-Curtis distance [22]. Correlations were assessed using vegan package mantel tests (version 2.5-6) and corroborated with the use of ANOSIM and PERMANOVA tests. Samples comparing two groups utilised Wilcoxon tests.

Results And Discussion

The present study was carried out in six fjords within New Zealand's Fiordland system, specifically Breaksea Sound, Chalky Inlet, Doubtful Sound, Dusky Sound, Long Sound, and Wet Jacket Arm, as described in Tobias-Hünefeldt et al., 2019. Analyses were divided into three categories: 1) a multi-fjord analysis comprising five of the tested fjords (excluding Long Sound), 2) a high resolution study along Long Sound's horizontal axis, and 3) a depth profile of Long Sound's deepest location. Total community composition (via 16S and 18S gene sequencing) and metabolic potential did not significantly covary across the five studied fjords (Mantel, $r < 0.01$, $P = 0.47$), Long Sound's horizontal transect (Mantel, $r < 0.01$, $P > 0.05$), or Long Sound's depth profile (Mantel, $r < 0.22$, $P > 0.05$). However, depth covaried with community structure for five studied fjords, across the horizontal transect at Long Sound, and along Long Sound's depth profile (Fig. 1, Figure S1-S3, Table S1). Microbial community changes observed along the horizontal axis were stronger between surface and 10 m communities (Mantel, Multifjord – $r = 0.21$, $P < 0.01$, Transect – prokaryotes $r = 0.47$, $P = < 0.01$, eukaryotes $r = 0.56$, $P < 0.01$), as opposed to along the horizontal axis of individual fjords (Mantel, Multifjord – $r = 0.08$, $P = 0.04$, Transect – prokaryotes $r = 0.21$, $P = 0.01$, eukaryotes $r = 0.13$, $P = 0.07$) (Figure S2-3). Additionally, significant differences in metabolic potential in response to depth were observed both across multiple fjords (Anosim: $R = 0.10$, $P = 0.03$) and along a transect from the entrance of the ocean to the head of Long Sound (Anosim: $R = 0.27$, $P = < 0.01$) (Fig. 1).

Across the five fjords (excluding Long Sound), surface samples were more metabolically active (i.e., average metabolic rate [AMR]) compared to the 10 m samples (Wilcox test, $W = 425$, $P < 0.01$). Samples

closer to the fjord head displayed increased metabolic rates (Wilcox test, W = 0, P < 0.01). Thus, metabolic variability varied with horizontal sampling location (Fig. 1b). While activity was not consistent along the length of Long Sound, surface samples in the low salinity layer were more metabolically active than those collected at 10 m (Figure S4). Heterotrophic production (via leucine incorporation) was not significantly correlated with microbial abundance within the five studied fjords and Long Sounds horizontal axis (Mantel – Multifjord r = 0.04, P = 0.22, Horizontal r = 0.04, P = 0.32). Along the depth profile, prokaryotic abundance and production were significantly correlated (Mantel, r = 0.60, P = 0.01) exhibiting a large drop in productivity from the surface to 10 m followed by a more gradual decrease.

To further explore the depth-associated changes we studied a high resolution depth profile of the deepest fjord (Fig. 2). We hypothesized that metabolic rate and diversity would be driven by marine snow linked to photosynthetic primary producers at the surface (e.g. phytoplankton and macroalgae; Fig. 2a) leading to a steady decrease in metabolic potential as resources were depleted with increases in depth. Any deviation altering the slow loss of metabolic potential would be linked to extraneous sources of nutrients uncoupled from surface activity (i.e. benthic influences, subsidies from land-based inputs). We observed a steady loss of metabolic diversity, and rate, from the surface to 100 meters (Fig. 2b, c), with sustained increases at depths past 100m. However, abundance did not follow the same pattern, and instead continuously decreased until 360 m (Fig. 2d). Abundance and metabolic changes over depth were associated with shifts in specific carbon utilization potential, where carbohydrate metabolism decreased from 12.7–6.8%, as carboxylic acid utilization increased from 12.0–29.5% from the surface to 360 m (Fig. 2e). This likely reflected the diminishing abundance of readily mineralizable substrates with depth, and the increase in recalcitrant sources of carbon and energy. Consistently, we also observed increases in phosphorylated chemical metabolism peaking at 40 and 360 m (Fig. 2e) as expected from utilization of phosphorous at the surface during blooms [23]. However, observed changes in metabolic potential did not reflect changes in prokaryotic or eukaryotic community composition, suggesting that while the community members were relatively consistent past a certain depth (i.e., 10 m for eukaryotes and 40 m for prokaryotes) the metabolic potential changed dynamically past 100 m, regaining peak metabolic potential with proximity to the bottom (Fig. 2f).

Our results demonstrate that metabolic potential and activity in fjords is linked to similar parameters as microbial community composition across surface or near surface sites. However, distinct selective pressures exist at aphotic sites which ultimately affect the link between phylogenetic and metabolic diversity. The observed patterns are contrary to the open ocean carbon pump paradigm and demonstrate that additional refractory sources of organic matter, including resuspension of terrestrial organic matter associated with benthic communities, are important contributors to microbial activity in fjords, which form a major marine biome worldwide (e.g. Patagonian, Scandinavian, Northeastern Pacific systems). We propose that this reflects the influence of the benthic microbial loop and incorporation and breakdown of terrestrial organic matter in fjordic sediments. Sediment resuspension can occur through a variety of abiotic [24,25] and biotic sources (known as bioturbation, [26]). The resuspension of organically rich sediments has previously been shown to increase microbial activity [27]. Observed patterns suggest that

resuspension could also be driven by bottom feeding organisms, increasing suspended organic matter and its utilization in near bottom habitats [28]. Therefore, organic matter sources influence the relationship between microbial communities and their metabolic potential.

Declarations

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Author contributions

SPTH performed the analysis and edited the manuscript. SRW contributed data, coordinated sampling, and edited the manuscript. FB conceived the study design, coordinated sampling, and edited the manuscript. SEM conceived the study design and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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_2021/.

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Figures

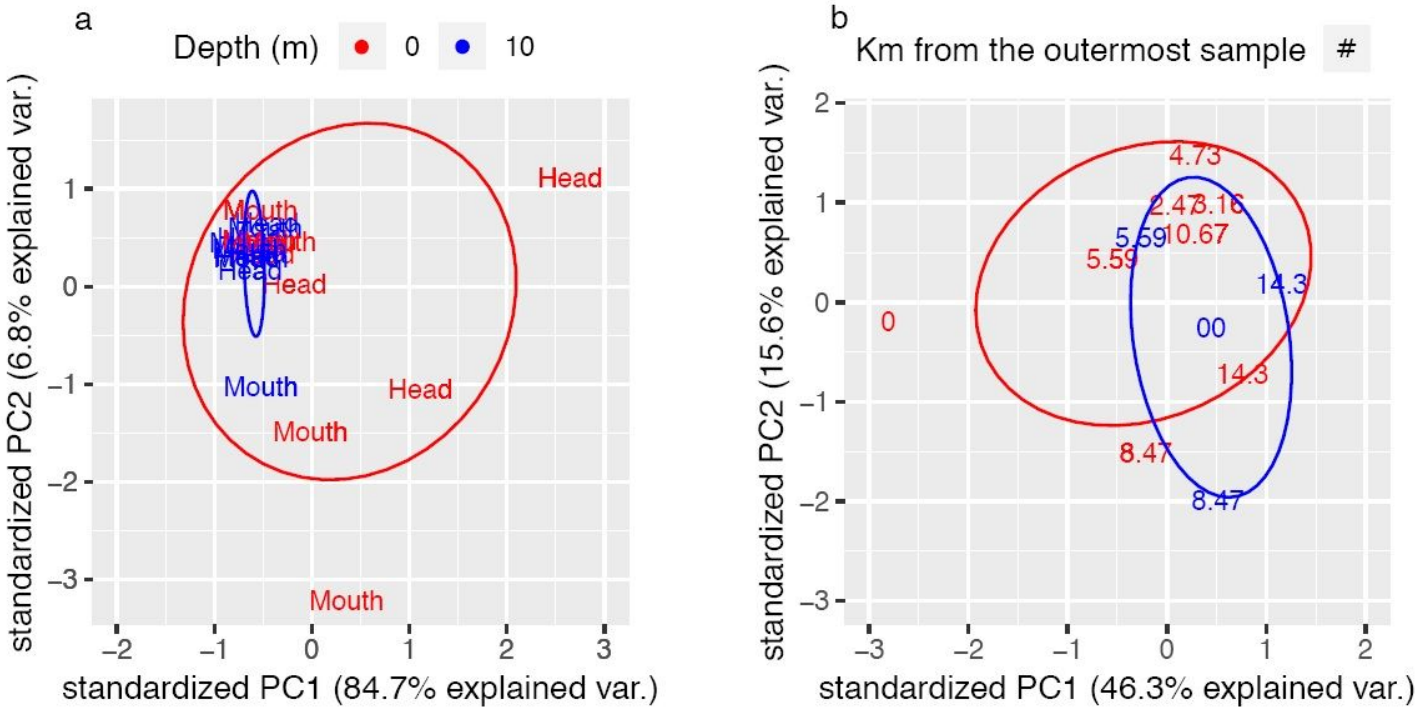

Figure 1

Biolog Ecoplate derived surface vs. 10 m PCA. Depth separated samples for Multifjord data (a) Long Sound's horizontal transect (b) calculated into a PCA plot. Text labels represent horizontal sample

location (head/mouth of the fjord [a], or being defined by the Km from the outermost sample [b]). Ellipses represent the 95% confidence interval.

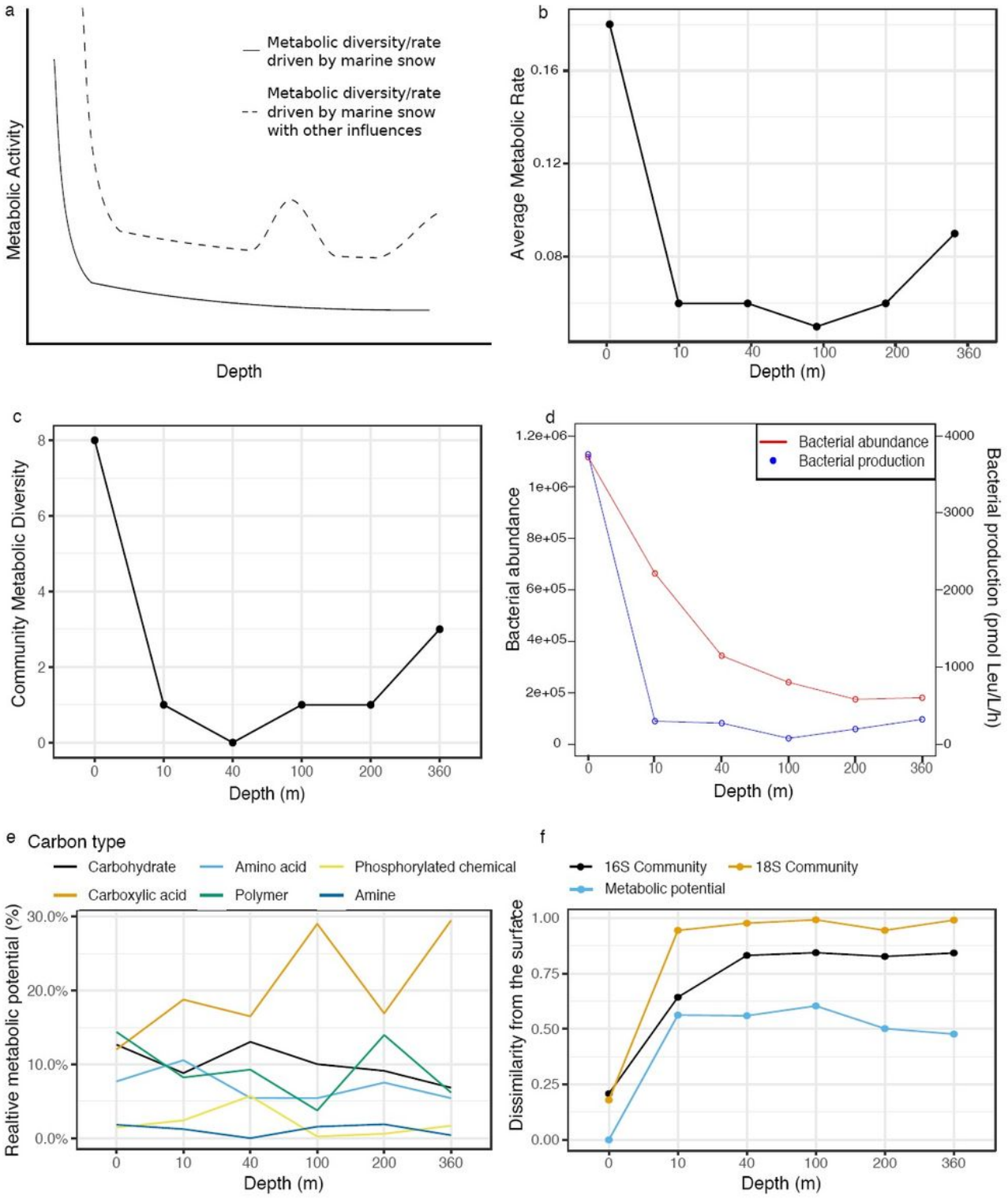

Figure 2

Benthic and surface influence on metabolic potential. Two metabolic potential scenarios are depicted (a), the metabolic rate and diversity when driven solely by photosynthetic production, and another model that accounts for additional benthic influences. Biolog Ecoplate plate derived Average Metabolic Rate (AMR,

b), Community Metabolic Diversity (c), and the relative metabolic potential (e) are also shown in addition to the bacterial abundance and productivity, and taxonomic and Biolog plate derived dissimilarity (Bray-Curtis) from the surface (f). Different colours represent carbon source groups (e; carbohydrates are blue, carboxylic acids are orange, amino acids are light blue, polymers are green, phosphorylated chemicals are yellow, and amines are dark blue), and Bray-Curtis dissimilarity data sources (f; the 16S community is black, 18S community is orange, and Biolog derived metabolic potential is light blue).

Supplementary Files

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