1 **Metabolic profiling suggests two sources of organic matter shape microbial activity, but**

2 **not community composition, in New Zealand fjords**

3

4 Sven P. Tobias-Hünefeldt[1], Stephen R. Wing[2], Federico Baltar[2,3,*] and Sergio E. Morales[1,*]

5

6 [1]Department of Microbiology and Immunology, University of Otago, PO Box 56, Dunedin

7 9054, New Zealand

8 [2]Department of Marine Science, University of Otago, PO Box 56, Dunedin 9054, New

9 Zealand

10 [3]Department of Limnology and Oceanography, Division of Bio-Oceanography, University of

11 Vienna, Althanstrasse 14, A-1090 Vienna, Austria

12

13 ***Correspondance:**

14 Dr. Sergio E. Morales, University of Otago, Department of Microbiology and Immunology,

15 720 Cumberland Street, North Dunedin, Dunedin 9054, New Zealand

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

17

18 Dr. Federico Baltar, Department of Limnology and Oceanography, Division of Bio-

19 Oceanography, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

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

21

22 ***Running title:*** Metabolic profiling of NZ fjords

23 ***Keywords:*** *Fjord; 16S; 18S; microbial community; Fiordland; metabolic diversity*

24

25 **Abstract**

26 Fjords are semi-enclosed marine systems with unique physical conditions that influence

27 microbial communities structure. Pronounced organic matter and physical condition gradients

28 within fjords provide a natural laboratory for the study of changes in microbial phylogeny

29 and metabolic potential in response to environmental conditions (e.g. depth). In the open

30 ocean new production from photosynthesis supplies organic matter to deeper aphotic layers,

31 sustaining microbial activity. We measured the metabolic diversity and activity of microbial

32 communities in fjords to determine patterns in metabolic potential across and within fjords,

33 and whether these patterns could be explained by community composition modifications. We

34 demonstrated that metabolic potential and activity are shaped by similar parameters as total

35 (prokaryotic and eukaryotic) microbial communities. However, we identified increases in

36 metabolic diversity and potential (but not in community composition) at near bottom

37 (aphotic) sites consistent with the influence of sediments in deeper waters. Thus, while

38 composition and function of the microbial community in the upper water column was likely

39 shaped by marine snow and sinking POM generated by new production, deeper sites were

40 strongly influenced by sediment resuspension of benthic organic matter generated from this

41 or other sources (terrestrial, chemoautotrophic, microbial carbon loop), uncoupling the

42 community composition and function dynamics.

43

44

45 **Introduction**

46 Fjords are unique environments, representing modified marine ecosystems mixing

47 freshwater, terrestrial and marine inputs. As such, influences on structure and function of

48 microbial communities are linked to changes in environmental conditions associated with

49 each input, including alternate organic carbon sources (e.g. tannins, terrestrial, marine and

50 freshwater sources), as well as modified salinity, nutrient, and light environments (Mckee et

51 al., 2002; Pulchan et al., 2003; Cui et al., 2016). Moreover, due to these strong environmental

52 gradients, fjords are ideal natural laboratories to study marine microbial communities and the

53 controls of their phylogenetic and functional diversity. However, the energy sources

54 supporting primary production and heterotrophic activity in fjords, and how they change in

55 correlation to observed community changes, remain poorly defined. In open ocean systems

56 primary productivity by surface phytoplankton mediates the downward flux of particulate

57 carbon, transferring energy to aphotic zones. This unidirectional transfer of carbon through

58 microbial/biological biomass from surface waters to deeper layers is termed the biological

59 carbon pump (Jiao et al., 2010; Jiao and Zheng, 2011; Legendre et al., 2015). This process is

60 also expected to dominate in fjords where carbon is predominantly linked to phytoplankton

61 production (Albright et al., 1986; Amy et al., 1987; Alldredge et al., 2002), sustaining a

62 significant portion of heterotrophic respiration (Iturriaga and Hoppe, 1977). Nevertheless,

63 fjord benthic community studies have demonstrated that microbial reworking of refractory

64 organic matter from terrestrial sources is an additional important source of carbon to deep

65 communities (McLeod and Wing 2007, McLeod and Wing 2009, McLeod Wing and Skilton

66 2010). Despite this, we lack an integrated view of microbial metabolic potential across fjords

67 and specific information about microbial populations possibly linked to them, providing a

68 mechanistic understanding of their selection. This limits our understanding of how this

69 ecosystem is sustained and shaped.

70   We previously examined the patterns in microbial community composition relative to

71   variability in environmental factors among fjords in the New Zealand Fiordland system

72   (Tobias-Hünefeldt et al., 2019). But links between patterns in phylogenetic and functional

73   diversity in these fjords remained unaddressed. In the present study we utilised functional

74   potential profiling (via Biolog Ecoplates), bacterial abundance, heterotrophic production (via

75   ^{3}H-leucine incorporation) and prokaryotic/eukaryotic community composition (via 16S and

76   18S rRNA amplicon sequencing) to compare community metabolic diversity and potential,

77   and how it related to known drivers of microbial community changes across six different

78   fjords in New Zealand. We found that community metabolic potential and diversity at surface

79   sites follow similar patterns to those observed when examining whole community

80   composition. However, a high resolution analysis along a depth profile of a fjord indicates

81   two potential drivers of metabolic diversity and potential (i.e. vertical transfer of carbon via

82   suspended particulate organic matter through the biological carbon pump, and resuspension

83   of organic matter from sediments linked to the benthic microbial loop and terrestrial carbon

84   sources).

85

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, utilising an identical sampling scheme as described in (Tobias-Hünefeldt et al., 2019). Analyses were divided into three categories, a multi-fjord analysis comprising five of the tested fjords (excluding Long Sound), a high resolution study along Long Sound's horizontal axis, and a depth profile of Long Sound's deepest location. Total community composition (via 16S and 18S 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 both metabolic potential and community structure among all fjords, across the horizontal transect at Long Sound, and along Long Sound's depth profile (Figure 1, Figure S1-S3, Table S1). Significant differences in metabolic potential with depth were observed both across multiple fjords (Anosim: R= 0.10, P value= 0.03) and along a transect from the entrance of the ocean to the head of Long Sound (Anosim: R= 0.27, P value= <0.01) (Figure 1). Microbial community changes 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 horizontal location (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).

Across the five fjords (excluding Long Sound), surface samples displayed higher metabolic potential (i.e., average metabolic rate [AMR]) compared to 10 m samples (Wilcox test, $W = 425$, $p = <0.01$) with samples from the fjord's head having a higher rate in general (Wilcox test, $W = 0$, $p = <0.01$). Horizontal sampling location affected the observed variance,

110   with higher variability in metabolic potential observed in samples collected near the fjord

111   entrance (Figure 1 b, Table S1). While activity was not consistent along the length of the

112   longest fjord, a sustained elevated activity was seen at surface compared to 10 m depths

113   (Figure S4). Heterotrophic production (via leucine incorporation) was not significantly

114   correlated with microbial abundance within the five studied fjords and Long Sounds

115   horizontal axis (Mantel – Multifjord r = 0.04, p = 0.22, Horizontal r = 0.04, p = 0.32),

116   consistent with either differences in grazing pressure between locations or a small proportion

117   of cells driving a large portion of the productivity. Along the depth profile, prokaryotic

118   abundance and production were significantly correlated (Mantel, r = 0.60, p = 0.01); however

119   clear differences were present, such as a more gradual difference in abundance compared to

120   the large drop in productivity from the surface to 10 m.

121   To further explore these depth linked changes we focused on a high resolution depth

122   profile of the deepest fjord. We hypothesized that metabolic rate and diversity would be

123   driven by marine snow linked to photosynthetic primary producers at the surface (e.g.

124   phytoplankton and macroalgae) (Figure 2a) leading to a steady decrease in metabolic

125   potential as resources were depleted with increases in depth. Any deviation altering the slow

126   loss of metabolic potential would be linked to extraneous sources of nutrients uncoupled from

127   surface activity. We observed a steady loss of metabolic diversity, and rate, from surface to

128   100 meters (Figure 2 b, c), with a sustained increase past this point. The observed pattern was

129   consistent with measurements for bacterial production, but not abundance, that decreased

130   continuously until reaching equilibrium from 200 m onwards (Figure 2 d). These changes

131   were associated with shifts in specific carbon utilization potential, where carbohydrate

132   metabolism decreased from 12.7% to 6.8%, as carboxylic acid utilization increased from

133   12.0% to 29.5% (Figure 2 e). This likely reflected the diminishing abundance of readily

134   mineralizable substrates with depth, and the increase in recalcitrant sources of carbon and

135   energy. Consistently, we also observed increases in phosphorylated chemical metabolism

136   peaking at 40 and 360 m (Figure 2e) as expected from utilization of phosphorous at the

137   surface during blooms (Tiselius and Kuylenstierna, 1996). However, observed changes in

138   metabolic potential did not reflect changes in prokaryotic or eukaryotic community

139   composition, suggesting that while the community members were relatively consistent past a

140   certain depth (10 m for eukaryotes and 40 m for prokaryotes) functional potential changed

141   dynamically past 100 m, regaining metabolic potential with proximity to the bottom (Figure 2

142   f) to a point closely resembling the metabolically active surface.

143   Our results demonstrate that while metabolic potential and activity in fjords is linked to

144   similar parameters as microbial community composition across surface or near surface sites,

145   distinct selective pressures exists at aphotic sites, ultimately affecting the link between

146   phylogenetic and metabolic diversity. The observed pattern is contrary to our initial

147   hypothesis and demonstrates that additional refractory sources of organic matter including

148   resuspension of terrestrial organic matter associated with benthic communities are important

149   contributors to microbial activity in fjords. We propose that this reflects the influence of the

150   benthic microbial loop and incorporation and breakdown of terrestrial organic matter in

151   fjordic sediments. Sediment resuspension through either wave or wind action (Pickrill, 1987;

152   Christiansen et al., 1992), as well as advection and biological activity provides mechanisms

153   for increased availability to the microbial community in the deep water column. Sediment

154   resuspension is known to increase metabolic activity of microbes (Flindt and Kamp-Nielsen,

155   2017) which is likely an important process in this deep water site where loose sediments are

156   organically rich reflecting suspended particulate matter [large amounts of fibrous woody

157   material, finer indeterminate organic plankton, faecal pellets with a small terrestrial

158   influence] (Pickrill, 1987; Pickrill, 1993). The observed pattern suggests that resuspension

159   could also be driven by bottom feeding organisms that can resuspend sediments, increasing

160   suspended carbon and its utilization in near bottom sites (Yahel et al., 2008), influencing the

161   relation between marine diversity and the metabolic potential of marine microbes.

162

Acknowledgements

169

170   **Conflict of interest**

171   The authors declare no conflict of interest.

172

173   **Data availability**

174   The sequence data from this study have been deposited in NCBI under BioProject

175   PRJNA540153. All data generated and/or analysed during the study is available within the

176   GitHub repository, https://github.com/SvenTobias-Hunefeldt/Fiordland_2019b/.

177

178   Supplementary information is available at Frontiers in Marine Science Journal's website'.

179

180 **References**

181

182 Albright, L.J., McCrae, S.K., and May, B.E. (1986) Attached and free-floating

183 bacterioplankton in howe sound, british columbia, a coastal marine fjord-embayment. *Appl*

184 *Environ Microbiol* **51**: 614-621.

185 Alldredge, A.L., Cowles, T.J., MacIntyre, S., Rines, J.E.B., Donaghay, P.L., Greenlaw, C.F.

186 et al. (2002) Occurrence and mechanisms of formation of a dramatic thin layer of marine

187 snow in a shallow Pacific fjord. *Marine Ecology Progress Series* **233**: 1-12.

188 Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman,

189 D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search

190 programs. *Nucleic Acids Res*. **25**: 3389–3402.

191 Amy, P.S., Caldwell, B.A., Soeldner, A.H., Morita, R.Y., and Albright, L.J. (1987) Microbial

192 Activity and Ultrastructure of Mineral-Based Marine Snow from Howe Sound, British-

193 Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **44**: 1135-1142.

194 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et

195 al. (2010) correspondence QIIME allows analysis of high- throughput community sequencing

196 data Intensity normalization improves color calling in SOLiD sequencing. *Nat Publ Gr* **7**:

197 335–336.

198 Christiansen, C., Zacharias, I., and Vang, T. (1992) Storage, Redistribution and Net Export of

199 Dissolved and Sediment-Bound Nutrients, Vejle Fjord, Denmark. *Hydrobiologia* **235**: 47-57.

200   Cui, X., Bianchi, T.S., Savage, C., and Smith, R.W. (2016) Organic carbon burial in fjords:

201   Terrestrial versus marine inputs. *Earth and Planetary Science Letters* **451**: 41-50.

202   Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST.

203   *Bioinformatics* **26**: 2460–2461.

204   Fischer H, P.M. (1999) Use of the [(14)C]leucine incorporation technique to measure

205   bacterial production in river sediments and the epiphyton. *Appl Environ Microbiol* **65**: 4411–

206   4418.

207   Flindt, M.R., and Kamp-Nielsen, L. (2017) The influence of sediment resuspension on

208   nutrient metabolism in the eutrophic Roskilde Fjord, Denmark. *SIL Proceedings, 1922-2010*

209   **26**: 1457-1461.

210   Iturriaga, R., and Hoppe, H.G. (1977) Observations of heterotrophic activity on

211   photoassimilated organic matter. *Marine Biology* **40**: 101-108.

212   Jiao, N., and Zheng, Q. (2011) The microbial carbon pump: from genes to ecosystems. *Appl*

213   *Environ Microbiol* **77**: 7439-7444.

214   Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W. et al. (2010)

215   Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the

216   global ocean. *Nat Rev Microbiol* **8**: 593-599.

217   Legendre, L., Rivkin, R.B., Weinbauer, M.G., Guidi, L., and Uitz, J. (2015) The microbial

218   carbon pump concept: Potential biogeochemical significance in the globally changing ocean.

219   *Progress in Oceanography* **134**: 432-450.

220   Mckee, D., Cunningham, A., and Jones, K.J. (2002) Optical and hydrographic consequences

221   of freshwater run-off during spring phytoplankton growth in a Scottish fjord. *Journal of*

222   *Plankton Research* **24**: 1163-1171.

223   Pickrill, R.A. (1987) Circulation and Sedimentation of Suspended Particulate Matter in New-

224   Zealand Fjords. *Marine Geology* **74**: 21-39.

225   Pickrill, R.A. (1993) SEDIMENT YIELDS IN FIORDLAND. *Journal of Hydrology (New*

226   *Zealand)* **31**: 39-55.

227   Pulchan, K.J., Helleur, R., and Abrajano, T.A. (2003) TMAH thermochemolysis

228   characterization of marine sedimentary organic matter in a Newfoundland fjord. *Organic*

229   *Geochemistry* **34**: 305-317.

230   Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013) The

231   SILVA ribosomal RNA gene database project: Improved data processing and web-based

232   tools. *Nucleic Acids Res*. **41**: 590–596.

233

234   Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., et al. (2017)

235   A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* **551**: 457–463.

236

237   Tiselius, P., and Kuylenstierna, M. (1996) Growth and decline of a diatom spring bloom:

238   Phytoplankton species composition, formation of marine snow and the role of heterotrophic

239   dinoflagellates. *Journal of Plankton Research* **18**: 133-155.

240   Tobias-Hünefeldt, S. P., Wing, S. R., Espinel-Velasco, N., Baltar, F., & Morales, S. E.

241   (2019). Depth and location influence prokaryotic and eukaryotic microbial community

242   structure in New Zealand fjords. *Science of the Total Environment*. 693, 133507

243   Yahel, G., Yahel, R., Katz, T., Lazar, B., Herut, B., and Tunnicliffe, V. (2008) Fish activity: a

244   major mechanism for sediment resuspension and organic matter remineralization in coastal

245   marine sediments. *Marine Ecology Progress Series* **372**: 195-209.

246

247

248   **Figure 1. Biolog Ecoplate derived surface vs. 10 m PCA.** Depth separated samples for

249   Multifjord data (a) Long Sound's horizontal transect (b) calculated into a PCA plot. Text

250   labels representing the horizontal location; either near the head/mouth of the fjord (a), or

251   being defined by the Km from the outermost sample (b). Ellipses represent the 95%

252   confidence interval. 150 µL of sample were utilised per Biolog Ecoplate well, and then

253   incubated for 7 days at 4˚C. Colour patterns assessed at OD A590 nm.

254   **Figure 2. Benthic and surface influence on metabolic potential.** A model of purely surface

255   vs. surface and benthic influences is shown (a), the Biolog Ecoplate plate derived Average

256   Metabolic Rate (AMR, b), Community Metabolic Diversity (c), and relative metabolic

257   potential (e). Bacterial abundance and productivity (c), and taxonomic and Biolog plate

258   derived dissimilarity from the surface (f). Different carbon source groups were displayed in

259   various colours (carbohydrates are blue, carboxylic acids are orange, amino acids are light

260   blue, polymers are green, phosphorylated chemicals are yellow, amines are dark blue), as

261   well as the Bray-Curtis dissimilarity measures (16S community being black, 18S community

262   being orange, and Biolog derived metabolic potential being light blue). Communities were

263   sequenced using the Earth Microbiome protocol (Thompson et al., 2017), OTUs were

264   generated using QIIME (Caporaso et al., 2012), UCLUST (Edgar, 2010) and SILVA (Quast

265   et al 2013).

266   **Figure S1. Microbial beta-diversity of five fjords.** The fjord of origin, sample region, and

267   depth were used to identify sample origin. Dissimilarity was assessed using Bray-Curtis

268   distance matrices based on OTUs at 97% similarity.

269   **Figure S2. Prokaryotic beta-diversity across Long Sound.** Beta-diversity based on 16S data

270   for Long Sound's horizontal axis. Dissimilarity was assessed using Bray-Curtis distance

271   matrices based on OTUs at 97% similarity.

13

272

273   **Figure S3. Eukaryotic beta-diversity across Long Sound.** Beta-diversity based 18S data for

274   Long Sound's horizontal axis. Dissimilarity was assessed using Bray-Curtis distance matrices

275   based on OTUs at 97% similarity.

276

277   **Figure S4. Average Metabolic rate across Long Sound.** The average metabolic rate

278   (AMR) across Long Sound's horizontal axis, colour separating depth (red being surface and

279   blue 10 m).

a

Metabolic diversity/rate
driven by marine snow

Metabolic diversity/rate
driven by marine snow
with other influences

b

c

d

e Carbon type

Carbohydrate   Amino acid   Phosphorylated chemical
Carboxylic acid   Polymer   Amine

f

16S Community   18S Community
Metabolic potential