

¹ Linking microbial diversity to macrobiota diversity in eelgrass
² meadows

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¹⁰ **Introduction**

- ¹¹ • Climate change is altering the structure of ecological communities worldwide—in most systems
¹² we see a decline in diversity, but effects are not uniform—limited understanding of underlying
¹³ mechanisms and key environmental variables that contribute to community resilience versus those
¹⁴ that are reshaping communities
- ¹⁵ 1. To date considerable efforts to understand how diversity is changing globally
 - ¹⁶ – But assessments of diversity often focus on specific guilds or taxonomic groups
 - ¹⁷ – Rarely do they span organismal scales, such as between micro and macro scales, even
within a single system
 - ¹⁸ – Increasingly clear that interactions between microbiota and macrobiota play an important
role in shaping ecological communities and should be incorporated into assessments
of biodiversity
- ¹⁹ 2. Considerable research has now demonstrated the myriad of ways that microbial diversity
²⁰ shapes ecological communities and ecosystem services
 - ²¹ – shape the environment—nutrient cycling—metabolizing organic carbon and nitrogen
 - ²² – link organisms across trophic levels
 - ²³ – species interactions and symbiosis (McRoy and Goering 1974; Kirchman et al 1984—
Sanders-Smith 2020)
 - ²⁴ – microbial diversity is product of: host sp identity, space, and local envirt
 - ²⁵ – but still limited understanding of the complexity of interactions and the environmental
factors that shape them
- ²⁶ 3. Eelgrass communities are an excellent system for furthering our understanding of micro-
²⁷ macro relationships across scales (split into 2 paragraphs)
 - ²⁸ (a) General relationships between eelgrass and diversity:
 - ²⁹ – Farjalla et al 2012: community assembly differs across scales—envirt filters and
interactions shape animals but microbes are stochastic

36 – But in marine ecosystems hosts more prone to horizontal transmission—Turon et
37 al 2018; Russel 2019—Sanders-Smith2020; global scale find that microbial comm
38 mirror that of envirt (Fahimipour2017—Sanders-Smith2020); but at finer scales see
39 selection by host through exudates

- 40 – Seagrass release methanol and cellulose—food for microbes—and phenols which
41 deters colonization:
42 – Crump et al 2018—Sanders-Smith2020:microbes consume toxic byproducts—methanol
43 and ethanol—and produce enzymes that limit epiphyte overgrowth
44 – Previous studies found evidence that in seagrass animal-microbial systems covary
45 Yeager et al 2019, Bengtsson et al. 2017

46 (b) Summary of seagrass work around bc/Calvert

- 47 – Several studies have studied eelgrass community diversity in meadows in coastal
48 BC: meadows that are farther apart are more dissimilar—driven by environment—
49 temp and DO explained variation more than habitat traits (LAI)—but taxa specific
50 (Stark2020)
51 – Sanders-Smith2020: differences in microbial community struct among regions but
52 not water column; differences in diversity associated with new (lowest richness
53 higher dominance) and old leaves
54 – Segovia2020:2 years of data: however alpha diversity on seagrass than water, most
55 abundant microeuk = diatoms, copepods, gregarines, and rotifers; Bray-curtis and
56 Jaccard both showed more similar communities on seagrass than other biofilms and
57 seawater—found spatial structuring in core taxa and a strong effect of temperature
58 – Meadows can differ in their diversity spatially, but less is known how they change
59 over time—further our understanding of how these processes covary and what po-
60 tential drivers may shape community responses is unclear

61 4. Here we explore how changes in microbial diversity effect macrobial communities in both
62 time and space—exploring the potential for microbes to mediate environmental conditions
63 and underlie diversity changes across a community.

- 64 – two four-year time-series of microbial diversity based on DNA sequences for the prokary-
65 ote and microeukaryote communities swabbed from eelgrass and paired with diversity
66 of epifauna communities present on the eelgrass.
67 – Use a joint Bayesian model to explore how diversity in micro and macro communities
68 change over time and whether species interactions are correlated with environment cues,
69 such as temperature or differences in habitat structure.
70 – In addition to estimating overall trends in diversity in eelgrass communities, we also
71 tested for trends across sites.

72 Materials and Methods

73 1. General intro to study area

- 74 • Both microbial and macro data collection was conducted around Calvert Island in BC
- 75 • Hakai Lúxvbálís conservancy: low human impacted *Zostera marina* meadows
- 76 • Meadows = monoculture of *Zostera marina* with macroalgae and microalgal
- 77 • 5 regions each with 2 sites, except Choked which had 3 sites (Choked, GPS: 51.66825, -
78 128.11855 and Pruth, GPS: 51.395, -128.069), Goose (GPS: 51.92652, -128.45317), McMullin
79 (GPS: 52.06178, -128.41239) and Triquet (GPS: 51.80869, -128.24746) islands (Fig. 1)

80 2. Epifauna surveys:

- Epifauna surveys and collection of seagrass habitat traits were collected from 2014 to 2017
- Using SCUBA sampled six 0.25 m by 0.25 m quadrats established in a 15m by 30m array placed in the middle of the meadow
- Collected eelgrass, detritus, macroalgae and epifaunal invertebrates
- Prior to collecting eelgrass traits, rinsed blades to remove epifaunal invertebrates and passed through 500 μm sieve.
- Invertebrates preserved 95% EtOH and identified upon returning from the field using light microscopy (10x magnification)
- Species were identified to the lowest taxonomic level possible using manuals by Light and Smith manual (Carlton 2007) and Kozloff (1996)
- Additional domain expertise of the natural history of our surveys species was used to remove species known to predominantly reside in eelgrass sediment ($n = 17$ species)
- For additional detail of the applied methods, see....

3. Microbial surveys

- Collection and sequencing of microbial communities was conducted from 2015 to 2018
- Collected one shoot just outside each quadrat sealed underwater in a sterile plastic bag at the location of collection.
- Swabs were taken from the second oldest blade, from an area with minimal to no epibionts and 10 cm in length, rinsing the area for 10 seconds with filtered seawater (0.22 μm) to remove attached organisms, and swabbed using Puritan sterile swabs for 10 seconds.
- Swabs were placed in cryotube and placed in a -80°C freezer within 6 hours of sampling and until DNA extraction
- Extracted DNA from swabs using Qiagen Powersoil—htp 96 well DNA extraction Kit (Carlsbad, CA): V4 region of 18S rRNA gene was targeted for amplification using primers E572F: 5'-AYGGTATCTRATCRTCTYG-3' and PCR, library construction, and Illumina MiSeq amplicon sequencing were carried out at the Integrated Microbiome Resource Facility, Centre for Comparative Genomics and Evolutionary Bioinformatics at Dalhousie University Halifax, Canada using standard protocols (Comeau et al. 2011)
- 16S DNA extraction: MoBio PowerSoilVR -htp 96 well DNA extraction kit (Carlsbad, CA), V4 region of the 16S rRNA gene in Bacteria and Archaea was targeted for amplification using redesigned versions of the primers 515f/806r as described by Lemay et al. 2018a.
- Removed OTUs with fewer than 2 reads for that sample to minimize the impact of barcode switching, and OTUs with fewer than 250 total reads and samples with low read counts (<1,000 reads/sample)
- For additional detail, see Sanders-Smith et al. 2020 and Segovia et al. 2020 for methods for sequence data analysis.

4. Climate and habitat traits

- At time of epifauna sampling collected data on bed area, meadow depth, eelgrass leaf area index, biomass of eelgrass and epiphytes'
- Measured shoot leaf area and biomass: measured leaf length, width and blade number of five haphazardly selected representative shoots from each quadrat, multiplying the average blade area (m^2) per shoot by the shoot density (shoots per m^2)
- LAI: structural complexity of bed and amount of habitat available for colonization by epiphytes and invertebrates (Enriquez et al. 2019, Green and Short 2003): LAI = mean blade length X width X number of blades per shoot; multiplying number of shoots in quadrat

- Additional shoot selected for microepiphyte biomass, scraping biofilm with glass slide filtering onto GF/C filters, dried and weighed
- Eelgrass and macroalgae biomass: biomass was separated and dried in a desiccator oven at 60°C for 48 hours.
- Our was to use temperature data recorded as close to our sites and at as temporally fine scale as possible
- Combined available historic climate data for each site, pooling across the Hakai CTD and sensor netwrok data (CITE website)
- For a given site and year, the frequency of temperature measurements varied considerably, as such we averaged the temperature observations across the summer months for each site (with summer defined as May to August in a given year).

137 **Statistical Analysis**

138 **Microbe sub-model**

139 The sub-model describes the processes that shape the changes in microbial community diversity over
140 time for 1 to n sites in our dataset. We use hierarchical modeling to partition sources of variation
141 including measurement error, and site-level differences. In particular, we assume that a observed
142 change in diversity for site i , Y_{micro_i} follows a normal distribution.

$$y_{\text{micro}} = \alpha_{\text{grandMicro}} + \alpha_{\text{site}_i} + \beta_{\text{year}_i} \times \text{year} + \sigma^2 \quad (1)$$

$$\alpha_{\text{microSite}} = \alpha_{\text{grandMicro}} + \alpha_{\text{site}_i} \quad (2)$$

$$(3)$$

143 The latent parameter $\alpha_{\text{grandMicro}}$ represents a value that is independent of site, $\alpha_{\text{site},j}$ and site-level
144 offsets from that diversity value, σ^2 is measurement error. Of these parameters, $\alpha_{\text{microSite}}$ is shared
145 by the macro community sub-model.

146 **Macro sub-model**

147 The macro-community sub-model describes the processes that determine the community diversity for
148 1 to n sites in relation to the microbial diversity. We predict that macro community diversity will be
149 shaped by habitat structure, including the biomass of macroalgae, bed area, depth, and the distance
150 from the bed's edge. The diversity of the microbial community, however, may further mitigate the
151 effects of temperature, salinity, and biomass, such that such that an observation of micro diversity at
152 a given site i under set g of temperatures, salinity, and seagrass biomass ($\text{temp}_j, \text{salinity}_j, \text{biomass}_j$)
153 contributes to observations of macro diversity, $Y_{\text{macro}_{i,j}}$ (which we z -scored to allow direct comparison
154 of cues).

$$\beta_{\text{temp}} = \alpha_{\text{temp},j} + \beta_{\text{micro.temp}} \cdot \alpha_{\text{microSite},j} \quad (4)$$

$$\beta_{\text{LAI}} = \alpha_{\text{LAI},j} + \beta_{\text{micro.LAI}} \cdot \alpha_{\text{microSite},j} \quad (5)$$

$$y_{\text{macro}} = \alpha_{\text{grandMacro}} + \alpha_{\text{site}_j} + \beta_{\text{year}_j} \cdot \text{year} + \beta_{\text{temp}_j} \cdot \text{temp} + \quad (6)$$

$$\beta_{\text{LAI}_j} \cdot \text{LAI} + \beta_{\text{depth}_j} \cdot \text{depth} \quad (7)$$

155 **Beta diversity**

$$y_{\text{beta}} \sim \text{Normal}(\mu, \sigma^2) \quad (8)$$

$$y_{\text{micro}} = \alpha_{\text{grandMicro}} + \alpha_{\text{site}_i} + \beta_{\text{year}_i} \times \text{year} + \sigma^2 \quad (9)$$

$$(10)$$

156 **Results**

- 157 • Summary of community composition and characteristics:

- 158 – Identified 3933 unique taxa of prokaryote and 1143 microeukaryote species.
159 – Across all sites, sampled 58373 invertebrates from 107 taxa.

160 – Across the four years of sampling, the number of individual epifauna sampled per 0.25 m^2
161 of seagrass meadow varied considerably, from 122 individuals sampled in 2015 in Goose SE
162 to 9393 individuals sampled in 2014 at our Triquet S site.

163 – Across all sites, the five most abundant taxa (*Caprella.californica*, *Caprella.laeviuscula*,
164 *Lacuna.vincta*", *Porcellidium*, *Gammaridea*) represented 60% of invertebrates collected,
165 although abundance was not consistent across all sites or years.

166 – Many species were not present at each site: only 11 taxa were present at each site, but 43%
167 of species were present at five or more sites.

168 • Changes in alpha diversity over time:

169 – In modelling the relationship between prokaryote diversity and epifauna: we found a slight
170 decline in prokaryote diversity over time (-0.17, 90% UI: -0.3, -0.01), but an increase in
171 epifauna diversity over time (0.26, 90% UI: 0.01, 0.48). These estimates remained stable
172 when the model was run with the last three years of data (results not shown).

173 – In trying to better understand the pathways through which microbial diversity may influence
174 epifauna diversity, we found no evidence that the observed changes in prokaryote diversity
175 interacting with changes in local temperature ($\beta_{micro,temp}$) or differences in eelgrass leaf
176 area ($\beta_{micro,LAI}$) to drive the observed increase in epifauna diversity.

177 – In modelling of the relationship between microeukaryote diversity and epifauna diversity, we
178 observed no change in microeukaryote diversity over time and similarly that temperature,
179 eelgrass leaf area had no influence on how species are interacting across these organismal
180 scales. and depth are not driving the overall increase in epifauna diversity we observe.

181 • In addition to finding these overall trends in diversity, we found some variation in the magnitude
182 and direction in which diversity is changing at our various sites

183 – The negative relationship estimated for prokaryote diversity across sites was driven by the
184 declines observed at four of our study sites, while the remaining five communities showed
185 no change

186 – While we observed no overall change in microeukaryote diversity, our model does estimate
187 some changes at the site-level, with two of our most southern sites (Pruth pocket and
188 Choked inner) increasing in diversity, but the nearby Choked sandspit experienced a decline
189 in diversity.

190 – Finally, we observed increases in epifauna diversity at four of our sites, while the remaining
191 five sites showed no change.

192 – Contrary to our expectations, none of these shifts appear to be related to differences in
193 site-level temperature or eelgrass LAI ??

194 • NMDS plots:

195 – NMDS plots show a fair fit of the data (stress values range from 0.15-0.2)

196 – Prokaryotes and microeukaryotes both depict some clustering across sites, plots within
197 Choked and Pruth are more similar to themselves, while McMullin, goose and Triquet
198 exhibit a more similarity in their communities

199 – Microeukaryotes

200 – Epifauna:

201 **Disucssion**

- 202 1. Summary of primary findings: some changes in diversity, changes in site level diversity—other
203 alternate drivers not currently identified
- 204 2. Compare the trends in eelgrass diversity we observed to meadows globally
- 205 3. Our dataset relative to other existing diversity datasets
- 206 4. Site-level differences and how contrast with stable temporal trends
- 207 5. The advantages of our method and the broad application to linking biodiversity across scales

208 **Figures**

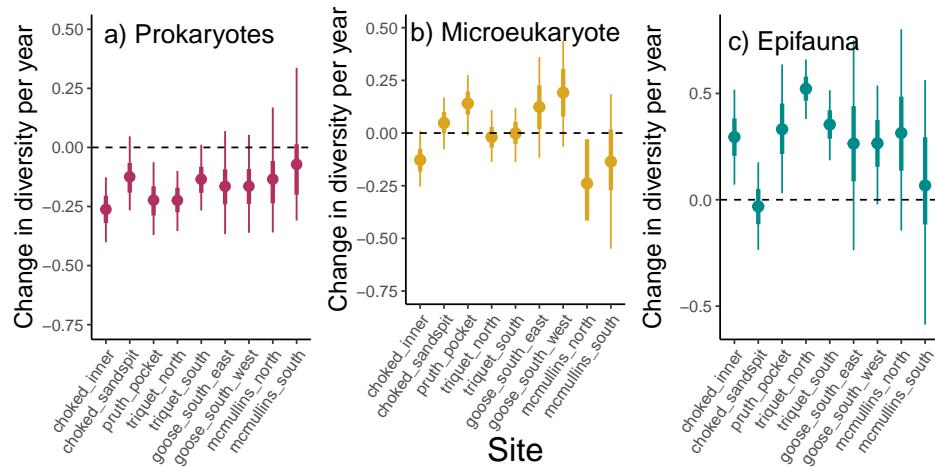


Figure 1: Site-level changes in Shannon diversity over time for the a) prokaryote communities, b) microeukaryote communities and c) epifauna communities. In general, our model estiamted a decline in the prokaryote diversity over time, while epifauna diveristy increased, but the microeukaryote community remained stable.

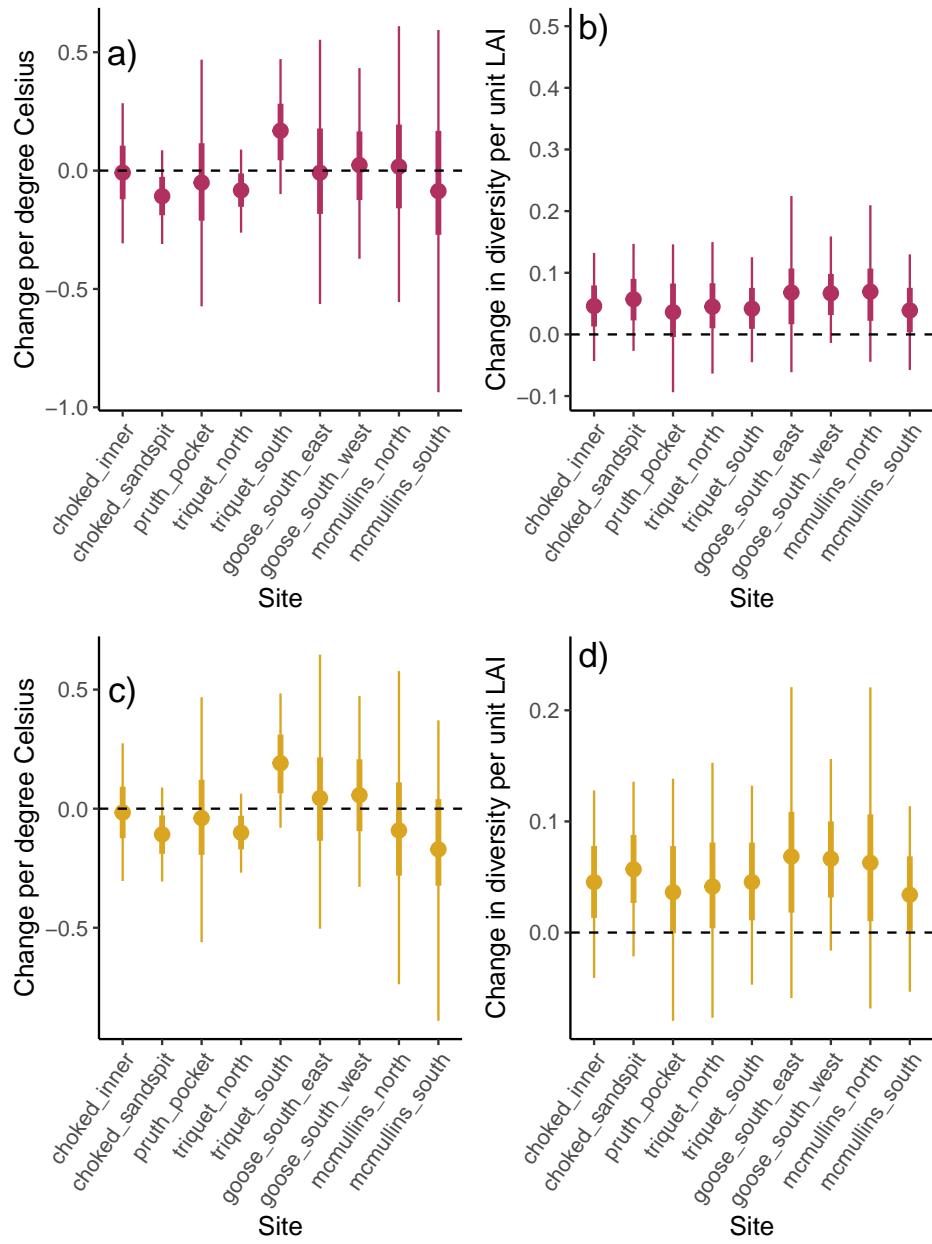


Figure 2: We found no relationship between prokaryote diversity and epifauna responses to a) temperature and b) leaf area index, or between microeukaryote diversity and c) temperature and d) leaf area index, with the exception of McMullins North, for which diversity declines as LAI increased in both models.

²⁰⁹ **Supporting Information**

Table 1: Summary output from a joint Bayesian model linking changes in Shannon diversity of prokaryote (16S) and macroepifauna diversity in relations to environmental conditions and habitat structure, with partial pooling for site. The model includes standardized environmental cues that are z -scored to allow comparisons to be made across parameters. In addition to the mean model estimates, both the 50%, and 90% uncertainty intervals are also reported.

	mean	5%	25%	75%	95%	n_eff	Rhat
mu_grand	4.25	3.96	4.15	4.37	4.52	1783.71	1.00
sigmaSt	0.28	0.02	0.12	0.40	0.68	312.63	1.00
muyear	-0.17	-0.30	-0.23	-0.12	-0.01	2402.75	1.00
sigmayear	0.12	0.02	0.06	0.17	0.29	1239.87	1.00
sigma_microy	0.66	0.59	0.62	0.68	0.73	3112.55	1.00
mu_grandM	1.15	0.71	0.99	1.32	1.58	1467.13	1.00
muyear2	0.26	0.01	0.18	0.35	0.48	2028.95	1.00
sigmayear2	0.30	0.13	0.19	0.36	0.58	1961.06	1.00
mutempSt	-0.15	-5.81	-2.32	1.83	6.28	2005.60	1.00
sigmatempSt	0.23	0.02	0.09	0.31	0.62	2047.55	1.00
muLAISt	0.08	-2.72	-0.57	0.56	3.27	700.78	1.00
sigmaLAISt	0.05	0.00	0.02	0.06	0.14	2845.40	1.00
muDepth	-0.03	-0.45	-0.19	0.13	0.40	2177.04	1.00
sigmaMacroSt	0.66	0.35	0.49	0.78	1.17	2104.04	1.00
sigmaMacro_y	0.27	0.23	0.25	0.28	0.31	4443.95	1.00
betaMicroxtemp	0.03	-1.48	-0.44	0.55	1.38	1999.65	1.00
betaMicroxLAI	-0.01	-0.75	-0.12	0.15	0.65	696.22	1.00

Table 2: Summary output from a joint Bayesian model linking changes in Shannon diversity of microeukaryotes (18S) and macroepifauna diversity in relations to environmental conditions and habitat structure, with partial pooling for site. The model includes standardized environmental cues that are z -scored to allow comparisons to be made across parameters. In addition to the mean model estimates, both the 50%, and 90% uncertainty intervals are also reported.

	mean	5%	25%	75%	95%	n_eff	Rhat
mu_grand	1.29	1.03	1.19	1.39	1.56	2025.22	1.00
sigmaSt	0.22	0.01	0.04	0.36	0.61	117.96	1.04
muyear	-0.00	-0.18	-0.06	0.07	0.17	1546.05	1.00
sigmayear	0.22	0.05	0.13	0.29	0.46	282.92	1.02
sigma_microy	0.55	0.49	0.53	0.58	0.62	3721.75	1.00
mu_grandM	1.17	0.72	1.00	1.33	1.61	1039.13	1.00
muyear2	0.27	0.01	0.18	0.35	0.49	1495.03	1.00
sigmayear2	0.30	0.13	0.20	0.37	0.58	1588.53	1.00
mutempSt	-0.54	-4.78	-1.59	0.34	3.87	1023.64	1.00
sigmatempSt	0.23	0.02	0.09	0.31	0.62	2177.12	1.00
muLAISt	-0.04	-2.37	-0.27	0.26	2.15	872.66	1.01
sigmaLAISt	0.05	0.00	0.02	0.06	0.13	2902.78	1.00
muDepth	-0.03	-0.47	-0.19	0.14	0.40	1915.93	1.00
sigmaMacroSt	0.67	0.35	0.48	0.79	1.19	885.37	1.00
sigmaMacro_y	0.27	0.23	0.25	0.28	0.30	3903.96	1.00
betaMicroxtemp	0.41	-3.03	-0.29	1.20	3.67	1006.95	1.00
betaMicroxLAI	0.07	-1.71	-0.15	0.24	1.87	873.19	1.01

Table 3: Summary output from a joint Bayesian model linking changes in Shannon diversity of prokaryote (16S) and macroepifauna diversity in relations to environmental conditions and habitat structure, with partial pooling for site, but with the first year of epifauna sampling removed. The model includes standardized environmental cues that are z -scored to allow comparisons to be made across parameters. In addition to the mean model estimates, both the 50%, and 90% uncertainty intervals are also reported.

	mean	5%	25%	75%	95%	n_eff	Rhat
mu_grand	4.26	3.99	4.16	4.37	4.53	1040.62	1.00
sigmaSt	0.19	0.00	0.03	0.30	0.56	112.59	1.03
muyear	-0.17	-0.31	-0.23	-0.11	0.01	936.11	1.00
sigmayear	0.13	0.02	0.06	0.17	0.31	734.51	1.01
sigma_microy	0.66	0.59	0.63	0.69	0.74	901.77	1.00
mu_grandM	0.15	0.07	0.12	0.19	0.24	1120.87	1.00
muyear2	0.05	0.01	0.03	0.07	0.09	1769.03	1.00
sigmayear2	0.02	0.00	0.01	0.03	0.06	1363.71	1.00
mutempSt	0.09	-4.66	-0.91	1.00	5.06	633.97	1.01
sigmatempSt	0.04	0.00	0.02	0.06	0.12	1827.49	1.00
muLAISt	0.01	-3.81	-0.48	0.49	4.05	619.22	1.01
sigmaLAISt	0.02	0.00	0.01	0.03	0.06	3128.49	1.00
muDepth	-0.01	-0.08	-0.03	0.01	0.05	1088.76	1.00
sigmaMacroSt	0.04	0.00	0.01	0.05	0.10	234.13	1.01
sigmaMacro_y	0.41	0.40	0.41	0.42	0.43	4254.89	1.00
betaMicroxtemp	-0.02	-1.18	-0.24	0.22	1.10	636.28	1.01
betaMicroxLAI	-0.01	-0.96	-0.12	0.11	0.87	622.61	1.01

Table 4: Summary output from a joint Bayesian model linking changes in Shannon diversity of microeukaryotes (18S) and macroepifauna diversity in relations to environmental conditions and habitat structure, with partial pooling for site, but with the first year of epifauna sampling removed. The model includes standardized environmental cues that are z -scored to allow comparisons to be made across parameters. In addition to the mean model estimates, both the 50%, and 90% uncertainty intervals are also reported.

	mean	5%	25%	75%	95%	n_eff	Rhat
mu_grand	1.29	1.06	1.20	1.38	1.52	1658.15	1.00
sigmaSt	0.08	0.00	0.01	0.06	0.42	45.41	1.13
muyear	-0.01	-0.21	-0.08	0.07	0.18	732.40	1.00
sigmayear	0.27	0.09	0.18	0.33	0.50	262.77	1.02
sigma_microy	0.56	0.50	0.53	0.58	0.62	2516.12	1.00
mu_grandM	0.15	0.07	0.12	0.19	0.24	1644.63	1.00
muyear2	0.05	0.01	0.03	0.07	0.09	1737.78	1.00
sigmayear2	0.02	0.00	0.01	0.03	0.06	1010.62	1.00
mutempSt	-0.02	-4.88	-1.33	1.23	4.76	1169.77	1.00
sigmatempSt	0.04	0.00	0.01	0.06	0.11	1637.07	1.00
muLAISt	0.13	-3.71	-0.85	0.93	4.36	771.78	1.01
sigmaLAISt	0.02	0.00	0.01	0.03	0.06	2602.05	1.00
muDepth	-0.01	-0.07	-0.03	0.01	0.05	1483.33	1.00
sigmaMacroSt	0.04	0.00	0.01	0.05	0.10	159.52	1.02
sigmaMacro_y	0.41	0.40	0.41	0.42	0.43	4324.66	1.00
betaMicroxtemp	0.00	-3.74	-0.96	1.03	3.75	1157.52	1.00
betaMicroxLAI	-0.10	-3.46	-0.74	0.65	2.91	779.11	1.01