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1 *Research article to Microbial Ecology*

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3 **Consistency and variation in the kelp microbiota: patterns**
4 **of bacterial community structure across spatial scales**

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38 **Key Words:** Holobiont, *Laminaria hyperborea*, *Saccharina latissima*, bacteria, core
39 microbiome

40 Abstract

41 Kelp species are distributed along ~25% of the world's coastlines and the forests they form
42 represent some of the world's most productive and diverse ecosystems. Like other marine
43 habitat-formers, the associated microbial community is fundamental for host and, in turn, wider
44 ecosystem functioning. Given there are thousands of bacteria-host associations, determining
45 which relationships are important remains a major challenge. We characterised the associated
46 bacteria of two habitat-forming kelp species, *Laminaria hyperborea* and *Saccharina latissima*,
47 from eight sites across a range of spatial scales (10s of metres to 100s of km) in the northeast
48 Atlantic. We found no difference in diversity or community structure between the two kelps but
49 there was evidence of regional structuring (across 100s km) and considerable variation
50 between individuals (10s of metres). Within sites, individuals shared few Amplicon Sequence
51 Variants (ASVs) and supported a very small proportion of diversity found across the wider
52 study area. However, consistent characteristics between individuals were observed with
53 individual host communities containing a small conserved "core" (8-11 ASVs comprising 25
54 and 32% of sample abundances for *L. hyperborea* and *S. latissima*, respectively). At a coarser
55 taxonomic resolution, communities were dominated by four classes (*Planctomycetes*,
56 *Gammaproteobacteria*, *Alphaproteobacteria* and *Bacteroidia*) that made up ~84 % of sample
57 abundances. Remaining taxa (47 classes) made up very little contribution to overall
58 abundance but the majority of taxonomic diversity. Overall, our study demonstrates the
59 consistent features of kelp bacterial communities across large spatial scales and
60 environmental gradients and provides an ecologically meaningful baseline to track
61 environmental change.
62

63 **Introduction**

64 Bacteria can be free living or form close associations with multicellular organisms. These
65 associated bacterial assemblages form part of the wider “microbiome” (along with fungi,
66 viruses and micro-eukaryotes), which strongly mediates the development and functioning of
67 the host organism [1–3]. The host-bacteria relationship also influences acclimation and
68 resilience to environmental stress and disruption can lead to dysbiosis, host disease and mass
69 mortalities [4–7]. It is therefore becoming increasingly apparent that macro-organisms and
70 their associated microbiota should be considered together as a single ecological unit known
71 as the “holobiont” [8].

72 Host-bacteria relationships are dynamic and complex as microbial community structure is very
73 responsive to stochastic and deterministic processes. These processes shift over
74 environmental, geographic and evolutionary scales and, as such, deciphering ecologically
75 meaningful associations from wider ecological noise is challenging. Attempts to achieve this
76 have focused around describing “common-core” communities and such studies have been
77 fundamental in identifying ecologically meaningful associations [9]. However, for most marine
78 habitat forming species the necessary large-scale studies characterising the host-bacteria
79 relationship over various environmental gradients are lacking.

80 Coastal marine ecosystems provide a wealth of ecological goods and services for human
81 society [10]. The structure and functioning of these ecosystems is strongly mediated by
82 habitat-forming foundation species (e.g., corals, mussels, seaweeds), which alter
83 environmental conditions and elevate local biodiversity [11, 12]. In turn, the functioning of host
84 foundation species is influenced by their associated microbiota and, as such, bacterial
85 communities likely play a vital role in the healthy functioning of the wider ecosystem [13]. Given
86 their importance, the role of foundation species in achieving conservation and restoration
87 goals is increasingly recognised [14–17] and in turn it is increasingly clear this will also be
88 dependent on a healthy host-bacteria relationship [18].

89 Kelps (large brown seaweeds belonging to the order Laminariales) are foundation species
90 distributed along around one-third of the world's coastlines [19, 20]. The forests they form are
91 fundamental wider ecosystem functioning and can represent some of the world's most
92 productive and diverse habitats [21, 22]. Like all seaweeds, the surfaces of kelp thalli
93 (hereafter 'plants') support dense bacterial communities, which are important in many aspects
94 of the host's biology, including metabolic function, nutrition and defence [23]. Moreover,
95 heterotrophic marine bacteria consume seaweed-derived compounds, providing a direct link
96 from primary to secondary production [24, 25] and may underpin a major pathway in coastal
97 nutrient cycling [26]. Therefore, describing the kelp-bacteria relationship is a critical step
98 towards understanding the wider coastal ecosystem. Recent studies have shown that bacterial
99 communities shift with host anatomy [27], across environmental gradients [28, 29], and
100 evidence from Australia demonstrated continental-scale structuring, high community turnover
101 and a small conserved core [30, 31]. However, our understanding in seaweeds still lags
102 considerably behind that of other benthic habitat formers (e.g., sponges and corals) and large-
103 scale multi-species studies are distinctly lacking for other kelp species and systems.

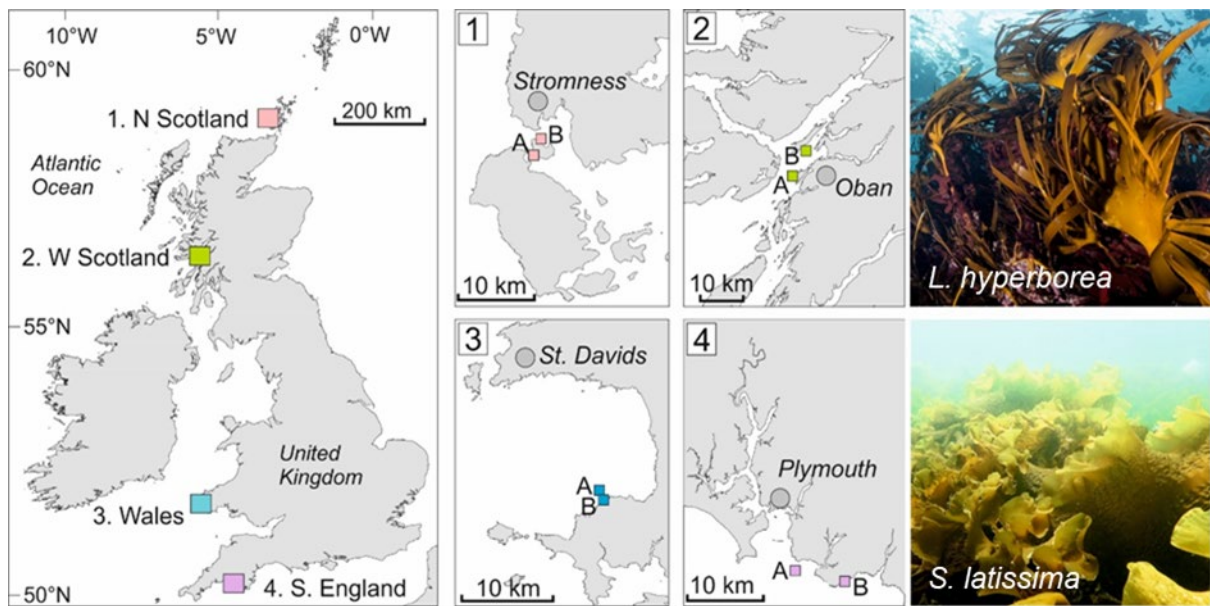
104 Along most of the Northeast (NE) Atlantic coastline, the kelps *Laminaria hyperborea*
105 (Gunnerus) Foslie 1885 and *Saccharina latissima* (Linnaeus) are the dominant foundation
106 species in shallow subtidal rocky habitats. Although these species have distinct environmental
107 requirements and occupy different niches, they co-exist in many coastal habitats [32] and
108 underpin productive and diverse ecosystems [33, 34]. Bacterial communities associated with
109 both species have been described previously but research has either been conducted in a
110 single location [35–37] or across a small-scale local environmental gradient [28]. Here, we
111 take a spatially structured approach to examine the bacteria-host relationship in both species
112 across a latitudinal gradient of $\sim 9^\circ$ in the United Kingdom, at scales from ~ 3 m to ~ 1000 km.
113 In doing so, we aim to determine i) at what scales host-bacteria relationships are structured
114 and ii) identify signatures of stability over spatial scales and between different hosts.

116 **Methods**

117 **Sampling approach**

118 Sampling followed a nested hierarchical approach based on four established study regions
119 with two sampling sites nested within each region (Figure 1). Adjacent regions were 180 – 500
120 km apart, spanning a gradient of 9° in latitude (~1000 km) [38]. Sites within each sampling
121 region were 2 – 10 km apart. The physical and biological attributes of these kelp forests sites
122 has been previously described [39, 40]. In summer (August/September) 2015, 7 – 8 mature
123 canopy-forming individuals of *Laminaria hyperborea* and *Saccharina latissima* were selected
124 at random at a depth of 2 – 4 m (below chart datum) from each site. Individual plants were
125 positioned at least 3 m and up to 10 m apart from one another, within the same continuous
126 patch of kelp habitat. Sampled individuals were brought to the surface where an area of 24
127 cm² of tissue was excised from the basal section of the blade, above the meristematic area
128 (i.e., ~ 10 cm above blade/stipe junction). Whilst seawater controls were not taken, studies
129 have consistently shown seaweed surface communities to be distinct from those of the wider
130 seawater environment [27, 29, 37, 41, 42]. The tissue was rinsed with sterilised seawater for
131 30 s to remove contamination of seawater DNA, and then scraped with a sterile razor blade.
132 The sampled biofilm was placed in a 1.5 ml Eppendorf and stored at - 80 °C. DNA was
133 extracted using Qiagen DNeasy Powersoil kits following the manufacturer's instructions.
134 Library preparation and sequencing (MiSeq, Illumina, San Diego, CA, United States) of the
135 V4 region of the 16S rDNA gene using primers (515f - GTGCCAGCMGCCGCGGTAA + 806r
136 - GGACTACHVHHHTWTCTAAT) was conducted by StarSEQ (StarSEQ GmbH, Mainz, DE)
137 following an optimised protocol of [43]. At least one negative PCR control was run on each
138 plate and demonstrated runs were free from contamination.

139



140

141 **Figure 1.** Map of study area showing position of four main study regions in the UK (left) and inset maps 1-4
 142 indicating positions of paired sites (A + B) within each region (centre). Representative individuals of the host kelps
 143 *Laminaria hyperborea* (top right) and *Saccharina latissima* (bottom right) are also shown.
 144

145 **Sequence processing**

146 All processing and analysis was conducted in the *r* statistical environment. Paired-end reads
 147 were processed according to the BIOCONDUCTER workflow [44]. Sequences were trimmed
 148 and truncated using the “filterAndTrim” function in DADA2 with the following parameters:
 149 truncLen, f = 240, r = 160; truncQ = 2; trimLeft, f = 20, r = 19, to remove primers and low quality
 150 reads. Amplicon Sequence Variants (ASVs) were resolved using DADA2 [44]. Chimeric
 151 sequences were removed using the “removeBimeraDenovo” function in DADA2. Sequence
 152 taxonomy was assigned using the RDP naïve Bayesian classifier against the SILVA release
 153 132 database [45] using the “assignTaxonomy” function in DADA2. Sequence read counts,
 154 taxonomic assignments and metadata were assembled as an object in the *r* package
 155 “PHYLOSEQ” and was used in downstream analysis [46]. Samples containing < 10,000 reads,
 156 taxa contributing < 0.01% of the reads in the dataset and ASVs identified as mitochondria or
 157 chloroplast were then removed from the PHYLOSEQ object. Sequence counts were then
 158 expressed as relative abundance (in proportion to the total sample count). Rarefaction curves
 159 of the processed reads were saturated, indicating good coverage of bacterial diversity (Figure

160 S1). Sequences are accessible through the EMBL database (accession no. PRJEB50679).
161 ASV table and metadata are available at (<https://doi.org/10.6084/m9.figshare.19453889.v1>).

162 **Statistical analysis**

163 To account for differences in sequence depth between samples in alpha diversity estimates,
164 the dataset was rarefied to the minimum sample depth, using the “rarefy_even_depth” function
165 in “PHYLOSEQ”. Alpha diversity for each sample was estimated through the Chao1 index
166 [47] implemented through the “estimate_richness” function in PHYLOSEQ. The Chao1 index
167 estimates ASV richness, and the standard error surrounding this estimate, based on the
168 observed number of ASVs, the observed number of ASVs occurring only once, and the
169 observed number of ASVs occurring only twice [47]. Alpha diversity was compared using a
170 three-way Analysis of Variance (ANOVA). Model factors consisted of Species (fixed factor;
171 two levels: *L. hyperborea*, *S. latissima*), Region (fixed factor; four levels: N Scotland, W
172 Scotland, Wales, S England) and Site (random factor; two levels: A, B). Differences in
173 community structure were determined using PERMANOVA [48] based on Bray-Curtis
174 dissimilarity and implemented through the “adonis” function in the package “VEGAN” [49].
175 Model design was the same as that for alpha diversity. The percent variation explained by
176 each model factor was quantified as the coefficient of determination (R^2), which is one minus
177 the ratio of the within-group sum of squares to the total sum of squares. Differences in
178 multivariate dispersion between assemblages were examined using the “betadisper” function
179 in “VEGAN”. A similarity of percentage (SIMPER) procedure was conducted in “VEGAN” to
180 determine which taxa contributed the most to observed dissimilarities.

181 To examine and define the “core bacterial community” we analysed each host kelp species
182 separately. We based the core at the ASV level and used a compositional dataset. There is
183 no consistent definition of a “core” in the literature with authors setting prevalence thresholds
184 from 50 – 100 %. Here, we used two tiers with prevalence thresholds of 95 % and 80 % (of
185 the total dataset with all regions included) in order to determine those taxa that a strictly

186 associated with each species, whilst also being comparable with recent studies in kelp [31]. In
187 both tiers, a relative sample abundance threshold of 0.1 % was used.

188 **Results**

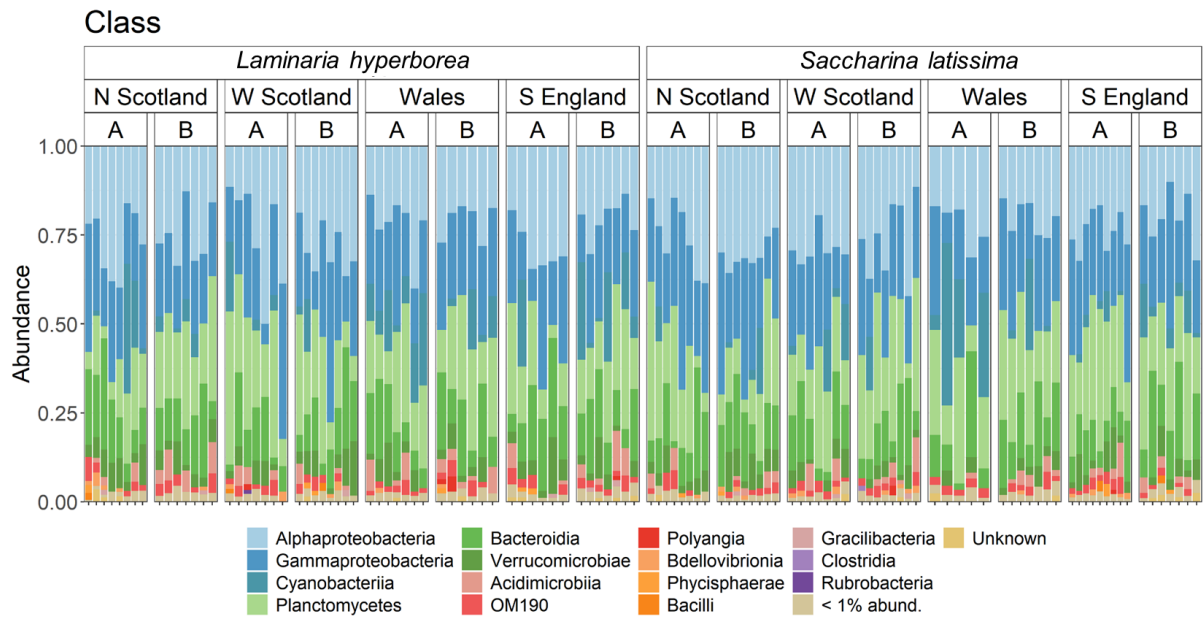
189 In total, we sampled bacterial communities from 115 kelps, which resulted in 4493603 paired
190 end reads with an average coverage of 39052 reads per sample. We identified 2824 ASVs
191 spanning 29 phyla, 52 classes, 121 orders and 236 families (Table S1, Figures 2 + S2 - S4).
192 The classes that made up the vast majority of bacterial abundance (~ 93%) were
193 Alphaproteobacteria (25.6%), Gammaproteobacteria (24.0%), Planctomycetes (21.0%),
194 Bacteroidia (13.1%), Cyanobacteriia (5.0%) and Verrucomicrobiae (4.4%) (Figure 2) (Table
195 S1). The relative contribution of these classes was remarkably consistent between species,
196 regions and sites (Figure 2). Even at a finer taxonomic resolution, the relative abundances of
197 bacterial taxa remained notably consistent across hosts and spatial scales (Figure S2 - S4).
198 At the family level, Pirellulaceae (19.4%), Hyphomonadaceae (15.7%), Saprospiraceae
199 (6.9%), Rhodobacteraceae (5.7%) and Flavobacteriaceae (5.5%) were the most abundant
200 (Figure S3, Table S1). Whilst the underlying taxonomy of ~ 30% of ASVs did not resolve down
201 to the genus level, 52% of sample abundance was made up of just twelve genera (Figure S4,
202 Table S1).

203 **Alpha Diversity**

204 *Shared ASVs*

205 Of the 2824 ASVs identified in this study (all regions combined), 1201 ASVs (42.5%) were
206 shared between both *L. hyperborea* and *S. latissima* (Figure S5). When *L. hyperborea* and *S.*
207 *latissima* were combined, 505 ASVs (17.8 %) were found across all study regions. When the
208 two species were examined separately, *L. hyperborea* shared 356 ASVs (17.2 %) and *S.*
209 *latissima* 366 ASVs (15.2 %) across all regions (Figure S5). Shared ASVs between individual
210 plants at the site level was far lower. When both kelp species were considered together
211 estimates ranged from 0.4 % (S England A) to 2.1 % (Wales A). When the two kelp species
212 were considered separately, estimates for *L. hyperborea* ranged from 1.0 % (W Scotland A)

213 to 3.9 % (Wales B), while estimates for *S. latissima* ranged from 1.1 % (S England A) to 4.9
 214 % (Wales A) (Table S2).



215

216 **Figure 2.** Relative abundance bacterial classes in the kelps *Laminaria hyperborea* (left) and *Saccharina latissima*
 217 (right) from four study regions (two sites A + B within each region) in the UK. Site locations can be seen in Figure
 218 1. Classes that made a contribution of < 1% to overall study wide abundance were collapsed into a separate
 219 category. "Unknown" represents taxa where underlying taxonomy was not resolved.

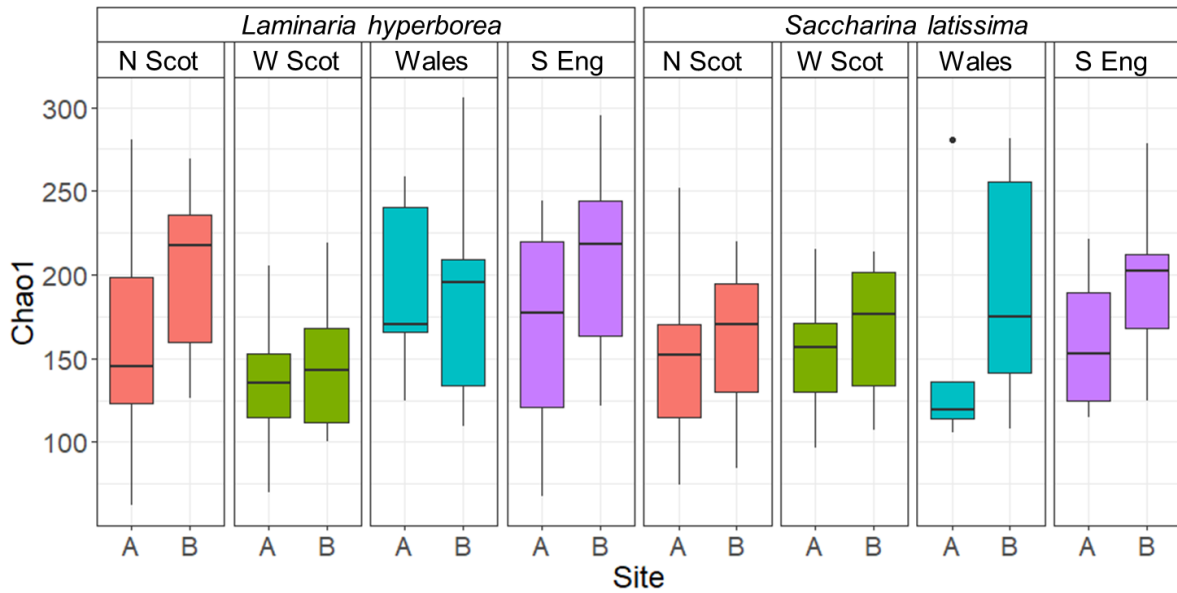
220

221 Bacterial ASV richness (Chao1 index) was not significantly different between Species or
 222 Regions or their interaction (Table 1). Our study wide estimate of mean richness for both
 223 species combined was 179.8 ± 5.2 S.E, which ranged from 152.7 ± 11.20 S.E. (W Scotland
 224 A) to 214.3 ± 17.5 S.E. (S England B). For *L. hyperborea*, mean richness was 182.9 ± 8.3 S.E.
 225 and ranged from 147.3 ± 16.9 S.E. (W Scotland A) to 217 ± 26.1 S.E. (S England B) (Figure
 226 3). For *S. latissima*, mean richness was 176.7 ± 7.4 and ranged from 158 ± 15.1 (W Scotland
 227 A) to 211 ± 25.3 (S England B) (Figure 3).

228

229 **Table 1.** Results of univariate (ANOVA) for alpha diversity and multivariate community structure (PERMANOVA)
 230 between Species, Regions and site variability within region.

	Alpha diversity (Chao1 index) (ANOVA)			Multivariate structure (PERMANOVA)			
	df	F	p	df	Pseudo-F	R ²	p
Species	1	0.66	0.42	1	0.81	0.01	0.64
Region	3	2.18	0.09	3	2.23	0.06	0.001
Species*Region	3	0.97	0.41	3	0.78	0.02	0.85
Species*Region(Site)	8	1.01	0.40	8	1.01	0.07	0.47
Residual	99					0.84	



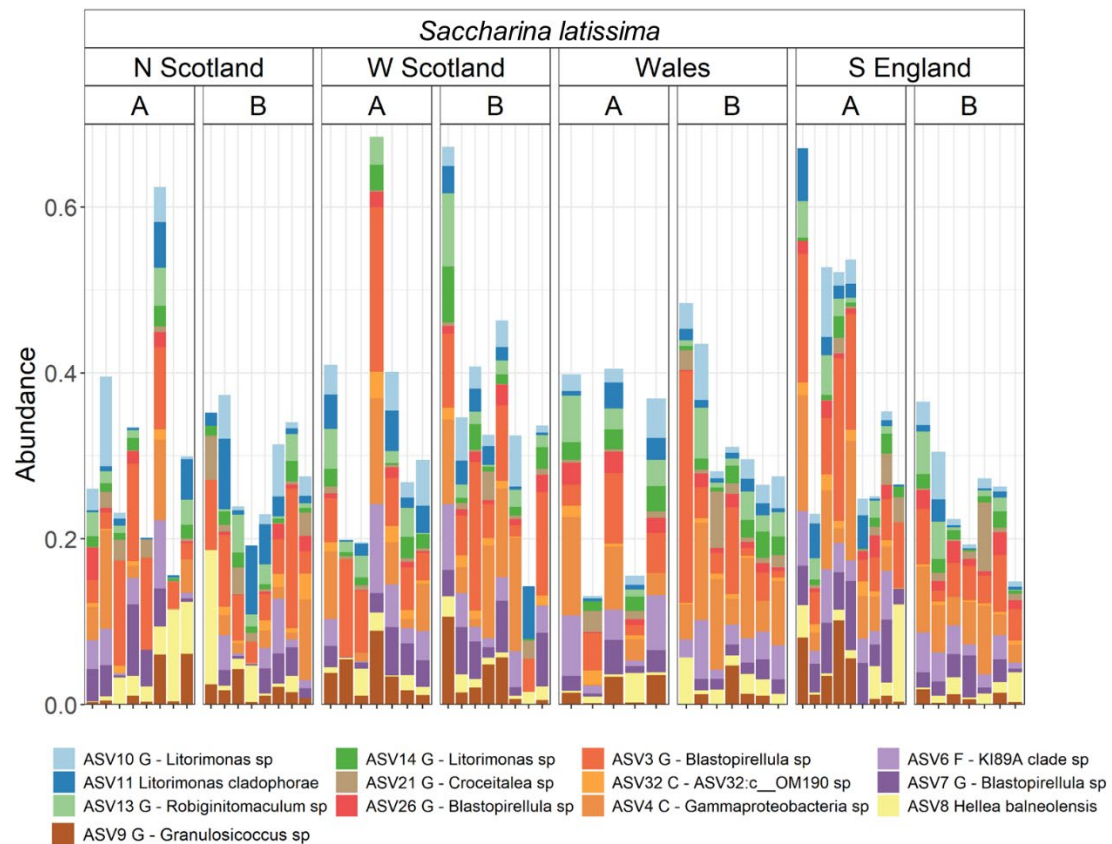
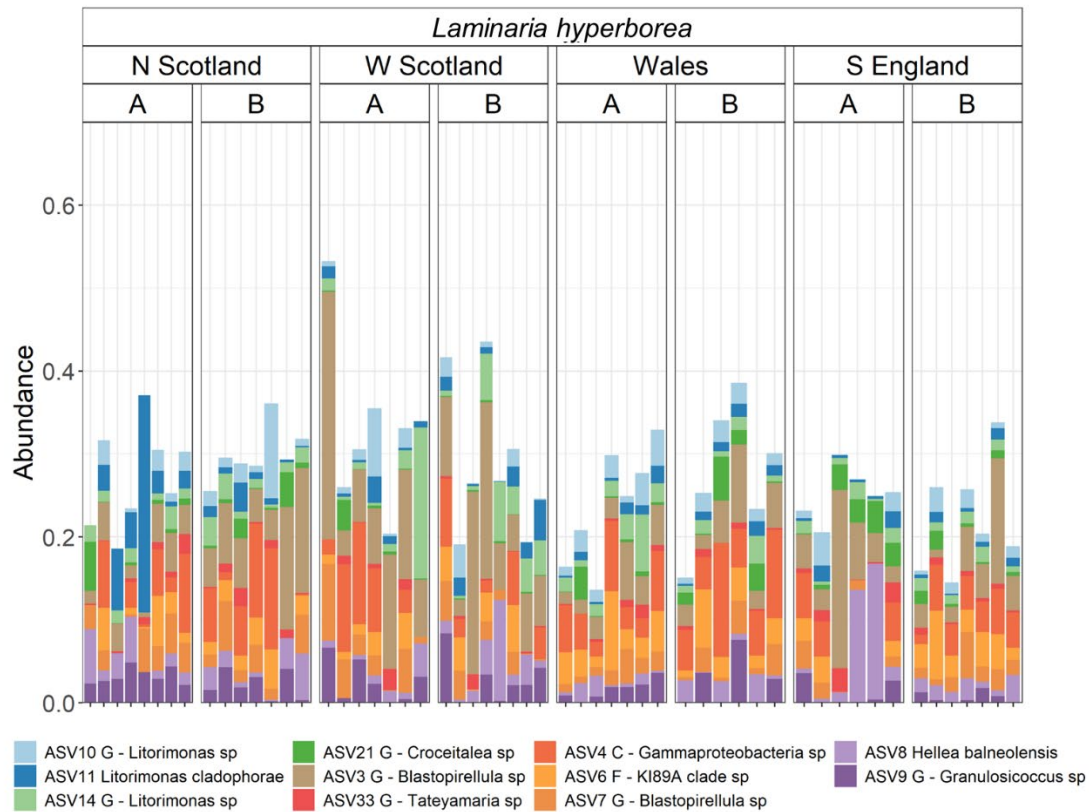
231

232 **Figure 3.** Box plots representing alpha diversity (Chao1 index) for bacterial communities associated with the kelps
 233 *Laminaria hyperborea* (left) and *Saccharina latissima* (right) from eight study sites in the UK. Site locations can be
 234 seen in Figure 1.

235

236 Core Community

237 Both species exhibited similar core bacterial communities. The first tier core (ASVs present in
 238 95% of samples), consisted of the same five ASVs for both kelp species. These were ASV3:
 239 *Blastopirellula* sp., ASV8: *Hellea balneolensis*, ASV11: *Litorimonas cladophorae*, ASV14:
 240 *Litorimonas* sp. and ASV21 *Croceitalea* sp. (Figure 4). Together, these five ASVs made up
 241 $13.8 \pm 0.9\%$ and $13.7\% \pm 0.9\%$ of the relative sample abundance for *L. hyperborea* and *S.*
 242 *latissima* respectively (Figure 4). The second tier core (ASVs present in 80% of samples)
 243 consisted of an additional six ASVs for *L. hyperborea* (11 total) and eight for *S. latissima* (13
 244 total). This wider core made up $25.4 \pm 0.9\%$ and $32.7 \pm 1.8\%$ of the relative sample abundance
 245 for *L. hyperborea* and *S. latissima* respectively (Figure 4).



246

247 **Figure 4.** Relative abundance of the core bacteria community (defined at taxa present in > 80% of samples at a
248 relative abundance > 0.1%) associated with the blade of the kelps *Laminaria hyperborea* and *Saccharina latissima*.
249 Abundance is expressed as proportion of entire sample. Letter represents lowest resolved taxonomic rank C =
250 Class, F = Family, G = Genus.

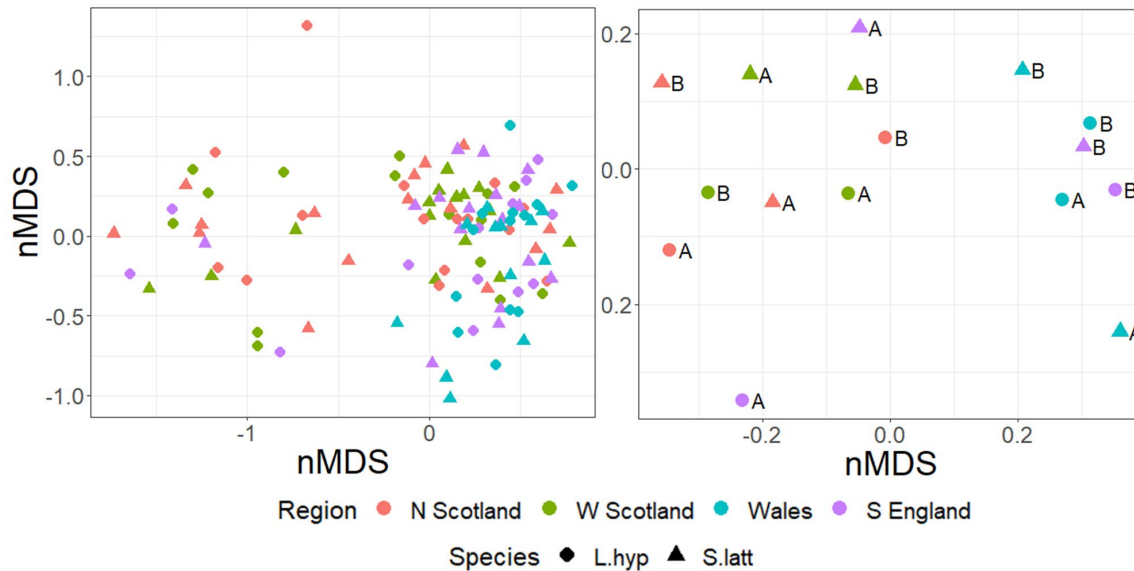
251 Community Structure

252 PERMDISP showed no significant differences in within-factor multivariate dispersion for either
 253 Species ($F_{(1, 119)} = 0.4, p = 0.5$) or Region ($F_{(3, 117)} = 0.4, p = 0.8$). PERMANOVA showed
 254 community structure varied significantly between regions but there was no significant effect of
 255 Species or within Region variability between sites. This pattern was consistent when analysis
 256 was performed at the higher taxonomic ranks of class and family (Table S3). Pairwise
 257 comparisons and nMDS visualisation showed Wales to be significantly different to N Scotland
 258 and W Scotland (Table 1, Figure 5). SIMPER analysis revealed the ASVs driving this
 259 difference were similar between W Scotland and N Scotland with only one different ASV in
 260 those contributing to 70% of observed dissimilarity (33 ASVs). Most notably, ASV5 –
 261 *Chroococidiopsis* sp. (Phylum; Cyanobacteria) and ASV4 - Gammaproteobacteria (Phylum;
 262 Proteobacteria) were found in higher abundances in Wales, while ASV3 - *Blastopirellula* sp
 263 (Phylum; Planctomycetes) was found in greater abundances in N and W Scotland (Table 2).
 264 Regional structuring was evident but the magnitude of variance explained (R^2) by each model
 265 component showed that residual scales were the major contributor to overall variability. Here,
 266 85% of variation remained unexplained (Table 1).

267 **Table 2.** SIMPER analysis on significant pairwise comparisons identified by PERMANOVA analysis. Presented
 268 taxa represent the top five that contributed most to the observed Bray-Curtis dissimilarities between comparisons.
 269 Letter represents lowest resolved taxonomic rank C = Class, F = Family, G = Genus.
 270

Taxa	Average abundance		Average dissimilarity between comparisons	Dissimilarity / SD	Contribution (%) to dissimilarity	Cumulative contribution to dissimilarity (%)
	N Scotland	Wales				
ASV 5 (G) <i>Chroococidiopsis</i>	0.021	0.069	0.037	0.77	5.1	5.1
ASV 4 (C) <i>Gammaproteobacteria</i>	0.037	0.059	0.024	1.40	3.3	8.4
ASV 3 (G) <i>Blastopirellula</i>	0.069	0.049	0.0223	0.96	2.8	11.2
ASV 20 (F) <i>Saprospiraceae</i>	0.007	0.031	0.016	0.78	2.6	13.8
ASV 24 (G) <i>Granulosicoccus</i>	0.008	0.032	0.015	0.71	2.1	15.9
	W Scotland	Wales				
ASV 5 (G) <i>Chroococidiopsis</i>	0.031	0.069	0.036	0.78	5.0	5.0
ASV 3 (G) <i>Blastopirellula</i>	0.094	0.049	0.033	1.01	4.7	9.7
ASV 4 (C) <i>Gammaproteobacteria</i>	0.047	0.059	0.024	1.44	3.5	13.2
ASV 20 (F) <i>Saprospiraceae</i>	0.010	0.031	0.016	0.81	2.3	15.5

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Figure 5. nMDS plots depicting the structure of bacterial assemblages associated with the kelps *Laminaria hyperborea* and *Saccharina latissima* from eight study sites in the UK. Symbols represent individual kelp communities (left) and site averages (right). Data are based on Bray-Curtis similarity. Site locations can be seen in Figure 1.

280 Discussion

281 Kelp are foundation species that play a disproportionately important role in the healthy
282 functioning of the wider reef ecosystem [21, 50]. However, the dynamic and variable nature of
283 its associated microbial community make it difficult to interpret. Here, we characterised this
284 relationship in two sympatric kelp species over a range of spatial scales in the UK. We found
285 communities were highly variable and differences between plants separated by 10s of metres
286 was often greater than between hosts, sites or regions. However, despite this high inter-
287 individual variation, consistencies and signals of stability were evident between the host
288 species and across large geographic scales.

289 Variation across scales

290 The bacterial communities associated with seaweeds have been shown to be structured over
291 a range of spatial scales, including microns [51], centimetres [27], tens [29], thousands [31]
292 and tens of thousands of kilometres [52]. In the most geographically extensive study to date,

293 Bonthold *et al.*, (2020) explicitly examined the relative importance of different spatial scales
294 across the distribution of the red alga, *Gracilaria vermiculophylla* (previously *Agarophyton*
295 *vermiculophyllum*), on associated microbiomes. They found hierarchal structuring across all
296 scales tested (10 - 10000 km) but processes operating at the site level (10's of kilometres) to
297 be the most important source of variation. Some structuring was observed at a regional scale
298 (100's km) but we did not observe any difference in community structure at the site level (10's
299 of km). Instead, we saw greatest variability between individual plants. This is in contrast to
300 other brown seaweeds, including kelp, that have found site level structuring [28, 29] and may
301 be due to a relatively similar environmental conditions across sites, or greater connectivity
302 overriding ecological drift.

303 The high levels of inter-individual variability is in contrast to bacterioplankton assemblages,
304 which show strong and robust geographical patterns in community structure [53–55]. Whilst
305 we do not have seawater comparisons, this suggests that host traits may play a stronger role
306 in community assembly than patterns in the wider environment. The high variability between
307 individuals also suggest a dominance of stochastic factors at the individual plant level. Burke
308 *et al.*, (2011) [56] proposed the “competitive lottery model”, originally developed for the
309 macroecology of reef fish [57], as a way of explaining the high variability between individual
310 algal hosts. Here, different subsets of bacteria, from the wider environmental species pool,
311 may have similar affinities for host traits and may provide similar suitable functions. However,
312 the final taxonomic community structure will be dependent on the randomness of the initial
313 colonisation. In our study, this may be exacerbated by the position of sampling on the host
314 itself. We sampled the meristem, which is the area of new growth in both hosts and may
315 represent an early stage in the bacterial succession trajectory. Indeed, previous studies have
316 found species richness to increase and community structure to shift on older parts of the kelp
317 blade [27, 29] and high variability between individuals where the meristem has been sampled
318 [31]. This means variability could decrease on older tissue as there is more time for
319 deterministic processes imposed by the host, site or region to take effect. However, given

320 growth of both kelp hosts is minimal in summer and autumn [32, 58] when our samples were
321 collected, our bacterial communities do not represent “newly settled” communities.

322 Whilst the vast majority of variation was unexplained, some regional structuring was also
323 evident. Specifically, differences were driven by bacterial communities in Wales being
324 significantly different to those in North and West Scotland. The prevailing climate of our Wales
325 sites is 2.5 °C (mean annual sea surface temperature) warmer than the northern cooler sites
326 of N and W Scotland and temperature has been found to impact various aspects of kelp
327 physiology and population structure across the same study site investigated here [38, 39].
328 However, given S England, which represents our warmest region, was not clearly
329 differentiated from the cooler regions it is unlikely temperature alone is responsible for this
330 structuring. A number of other regional scale factors have been associated with shifts in host-
331 bacteria community structure including variation in salinity [29], wave exposure [28], turbidity
332 [59], nutrient concentrations [31] and host genetic factors [60] and these could be responsible
333 for driving regional differences observed here.

334 **Signatures of Stability**

335 Despite high between-host variation, consistencies were observed across all the spatial scales
336 of our study. At a coarser taxonomic resolution, out of the 52 classes we observed, the vast
337 majority of sample abundance was constrained to *Alphaproteobacteria*,
338 *Gammaproteobacteria*, *Planctomycetes*, and *Bacteroidia*. Whilst we do not have appropriate
339 environmental controls (seawater and other biofilms) to make direct comparisons, the
340 consistently high abundance of *Planctomycetes* is interesting. This group have been found
341 enriched in many seaweeds around the world, including *S. latissima* and *L. hyperborea* [36,
342 56, 61–64]. *Planctomycetes* have high numbers of sulfatase genes that can degrade agars
343 associated with macroalgae providing them with resources [65]. The precise role of
344 *Planctomycetes* for the host remains speculative but their consistent association across all
345 scales tested here and more widely for seaweeds generally suggests they may play an

346 important role for the host. Ultimately, such an understanding will be gained as our knowledge
347 of Planctomycetal physiology increases and full genomes of taxa in this group become
348 available.

349 **Core community**

350 The “core community” concept aims to identify stable, functionally important taxa rather than
351 transient or opportunistic components of the community [66]. In this study, both kelp hosts
352 possessed a small core community that was similar in size and composition. The ASVs that
353 were present in > 80% of samples made up 25.4% (*L. hyperborea* – 11 ASVs) and 32.7% (*S.*
354 *latissima* – 13 ASVs) of the overall sample relative abundance, despite representing < 5 % of
355 the ASVs present in a typical plant. The stability of this core community contrasts with recent
356 attempts to characterise a common core in three species of green algae in the *Ulva* genus,
357 where taxonomic composition was too variable. However, such communities have been
358 effectively described in a range of other seaweeds [52, 67] and the size and composition is
359 comparable to estimates across large spatial scales in the kelp, *E. radiata*, in Australia [31].
360 Indeed, the core community associated with *E. radiata* is similar in size (15-55 taxa making
361 up 33-35% of relative sample abundance) and shares many taxa that form the core
362 communities of our two host species, including the genera *Blastopirellula* (Planctomycetes),
363 *Granulosicoccus* (Gammaproteobacteria) and *Hellea* (Alphaproteobacteria) [30, 31].

364 Many of our core taxa have been reported in association with seaweeds in systems and
365 species across the world [29, 36, 64, 67–70]. Such interspecific consistencies suggests that a
366 “core bacterial community” may encompass seaweeds more generally and particular taxa may
367 be associated with diverse host phylogenies even across large geographic scales. Whilst our
368 core community is based on taxonomy, the ecology and genome profiles of many ASVs
369 suggest they may be functionally important. Specifically, *Litorimonas* (3 core ASVs) may aid
370 in photosynthesis through oxygen detoxification and CO₂ evolution [71], while
371 *Granulosicoccus* are chemo-heterotrophic bacteria capable of reducing nitrate [68, 72].
372 However, further large-scale studies incorporating other hosts and a greater understanding of

373 their functional profiles are required before the ubiquity and utility of this core can be
374 determined.

375 Outside of the four dominant classes and core-ASVs associated with each host, the remaining
376 bacterial community was generally made up of classes with low diversity, abundance and only
377 appeared in a small number of samples. This highly variable constituent of the bacteria
378 community is likely reflective of both deterministic and stochastic processes operating within
379 the reef environment and the interaction between plants, other organisms and the surrounding
380 seawater. Shallow subtidal reef habitats are highly dynamic and influenced by a number of
381 factors that vary across multiple scales, including wave exposure, light and nutrient availability,
382 sedimentation rates and salinity fluctuations [73–75]. These factors may interact in a multitude
383 of ways to influence bacteria communities found at any given time or host. Future studies
384 using high-resolution *in situ* measurements of physical and biological variables will provide
385 insights into the drivers of bacterial community structure.

386 **Similarities between hosts**

387 We observed no difference in community structure between associated bacterial communities
388 of *L. hyperborea* and *S. latissima*, with these two hosts sharing 40% of observed ASVs. This
389 is similar to the 37% of bacterial taxa shared between eight sympatric kelp hosts in British
390 Columbia [42]. Moreover, many of the most diverse families observed in our study (e.g.,
391 Flavobacteriaceae, Saprospiraceae, Rhodobacteraceae) (Figure S3) have been identified as
392 seaweed generalists [76]. Therefore, it may not be surprising that our hosts share a large
393 proportion of bacteria taxa between them. The lack of structure may also be a product of
394 similarities in the chemical and physical properties of the both kelp hosts [77], reflective of their
395 recent evolutionary divergence [78]. Indeed, differences in bacterial community structure exist
396 between other sympatric species from the three different algal lineages (red, green and brown
397 algae) that have far greater evolutionary distance [63]. However, it is important to note that we
398 sampled the meristematic region, which represents an early stage in the colonisation and

399 development of bacterial communities [28] and, as such, interspecific differences may
400 manifest at later stages of bacterial succession.

401 In summary, kelp harbour thousands of bacterial associations but individual communities are
402 largely constrained to four taxonomic classes and have a small conserved core at the ASV
403 level. This was consistent across large spatial scales and between different host species and
404 may be a common characteristic of kelp bacterial communities more generally. Given host-
405 associated microbial communities are increasingly recognised for their role in mediating host
406 resilience to environmental perturbations, and kelps are threatened by a range of stressors,
407 these data provides critical insight into the stability of the healthy host-microbiome complex.
408 Future studies documenting how the breakdown of this relationship may impact host condition
409 may lead to robust microbial indicators of stress across large spatial scales.

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416 **Author Contribution**

417 PJM and DAS designed the experiment. NGK and JT conducted all laboratory work
418 and analysis. NGK lead the manuscript preparation and all authors contributed equally
419 to subsequent edits. All authors read and approved the final manuscript

420 **Data Accessibility**

421 Sequences are accessible through the EMBL database (accession no. PRJEB50679). ASV
422 table and metadata are available at (<https://doi.org/10.6084/m9.figshare.19453889.v1>)

423 **Statements and Declarations**

424 **Conflict of interest** The authors declare that they have no conflict of interest.

425 **Ethical Declaration** No approval of research ethics committees was required to accomplish
426 the goals of this study because experimental work was conducted on unregulated kelp
427 species.

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634 **Supplementary Information**635
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639**Table S1.** Relative abundance and number of ASVs (nASV) per class, order and genus of 121 kelp samples (*L. hyperborea* and *S. latissima* combined) from eight sites in the United Kingdom. Taxa shown represent those contributing over > 1% to overall sample abundance.

Class	Relative abundance	nASV
Alphaproteobacteria	25.6	519
Gammaproteobacteria	24.0	482
Planctomycetes	21.0	105
Bacteroidia	13.1	612
Cyanobacteriia	5	36
Verrucomicrobiae	4.4	126
Acidimicrobiia	2.1	26
OM190	2.0	63
Order		
Pirellulales	19.4	75
Caulobacterales	16.4	51
Chitinophagales	6.9	161
Flavobacteriales	5.7	283
Rhodobacterales	5.7	85
Granulosicoccales	5.4	21
Verrucomicrobiales	4.4	104
Pseudomonadales	4.2	155
Cyanobacteriales	4.1	16
Microtrichales	2.1	25
Thiotrichales	2.1	19
Arenicellales	1.8	33
Planctomycetales	1.6	29
Unknown	12.9	
< 1 %	7.4	
Family		
Pirellulaceae	19.4	75
Hyphomonadaceae	15.7	40
Saprospiraceae	6.9	157
Rhodobacteraceae	5.7	85
Flavobacteriaceae	5.5	199
Granulosicoccaceae	5.4	21
Xenococcaceae	4.1	8
Rubritaleaceae	3.4	73
K189A clade	3.1	16
Microtrichaceae	2.1	24
Thiotrichaceae	2.1	19
Arenicellaceae	1.8	33
Rubinisphaeraceae	1.6	24
Unknown	13	

< 1%	10.8	
Genus		
Blastopirellula	17.9	46
Litorimonas	7.6	12
Granulosicoccus	5.4	21
Chroococciopsis	3.4	3
Hellea	3.0	2
Sva0996 marine group	2.1	14
Roseibacillus	2.1	28
Cocleimonas	2.0	12
Robiginitomaculum	2.0	4
Arenicella	1.7	21
Octadecabacter	1.4	5
Croceitalea	1.3	3
Unknown	27.9	
<1%	22.4	

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Table S2. Shared ASVs between all host plants (*S. latissima* and *L. hyperborea*) at study region and sites in the United Kingdom.

<i>L. hyperborea</i> + <i>S. latissima</i>				
Region	Site	Shared ASVs	Total Site ASVs	%
N Scotland	A	4	987	0.40
N Scotland	B	5	963	0.51
W Scotland	A	5	846	0.59
W Scotland	B	5	1014	0.49
Wales	A	19	897	2.11
Wales	B	16	951	1.68
S England	A	4	964	0.41
S England	B	22	1046	2.10
<i>L. hyperborea</i>				
N Scotland	A	9	671	1.34
N Scotland	B	11	709	1.55
W Scotland	A	6	581	1.03
W Scotland	B	8	701	1.14
Wales	A	26	716	3.63
Wales	B	26	670	3.88
S England	A	10	615	1.62
S England	B	26	749	3.47
<i>S. latissima</i>				
N Scotland	A	8	700	1.14
N Scotland	B	10	644	1.55
W Scotland	A	17	558	3.04

W Scotland	B	8	694	1.15
Wales	A	24	485	4.94
Wales	B	25	662	3.77
S England	A	8	721	1.10
S England	B	27	735	3.67

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Table S3. Results of multivariate community structure (PERMANOVA) between Species, Regions and site variability within region. Analysis was performed on data agglomerated back to the taxonomic ranks of class and family/

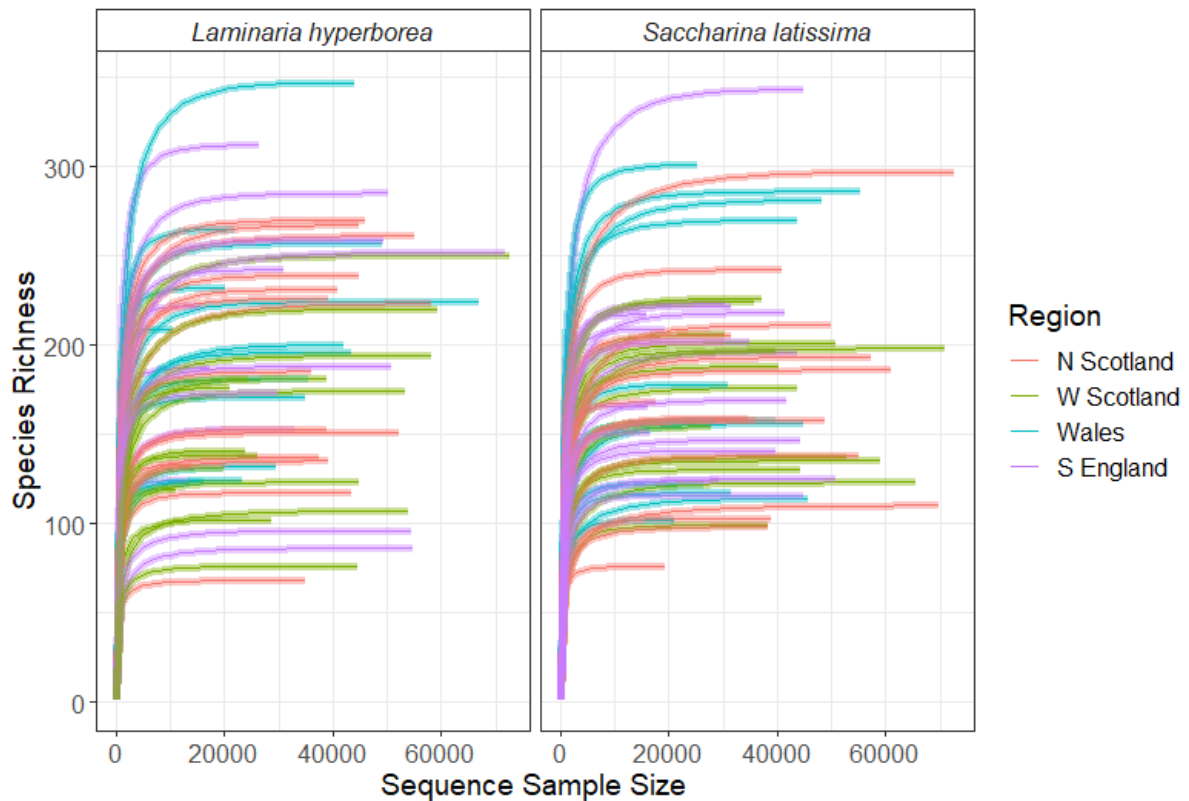
	df	Class		Family	
		F	p	F	p
Species	1	0.725	0.577	0.732	0.614
Region	3	1.789	0.05	2.20	0.005
Species*Region	3	0.631	0.765	0.724	0.797
Species*Region(Site)	8	1.207	0.205	1.016	0.452
Residual	99				

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Table S4. Pairwise comparisons of significant factors (Region) from multivariate PERMANOVA analysis. p values are with Bonferroni correction applied.

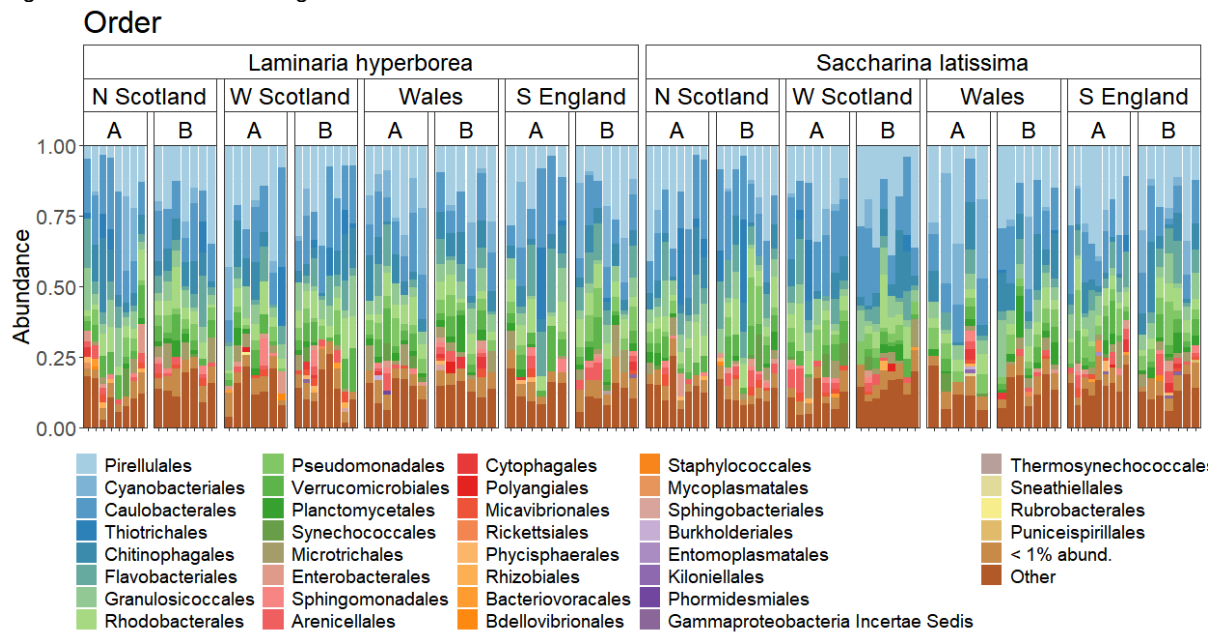
Comparison	df	Pseudo-F	R ²	p
W Scot vs N Scot	1	0.66	0.001	1.0
W Scot vs S Eng	1	1.85	0.03	0.228
W Scot vs Wales	1	4.04	0.07	0.013
N Scot vs S Eng	1	1.69	0.03	0.312
N Scot vs Wales	1	4.24	0.07	0.006
S Eng vs Wales	1	1.45	0.03	0.630

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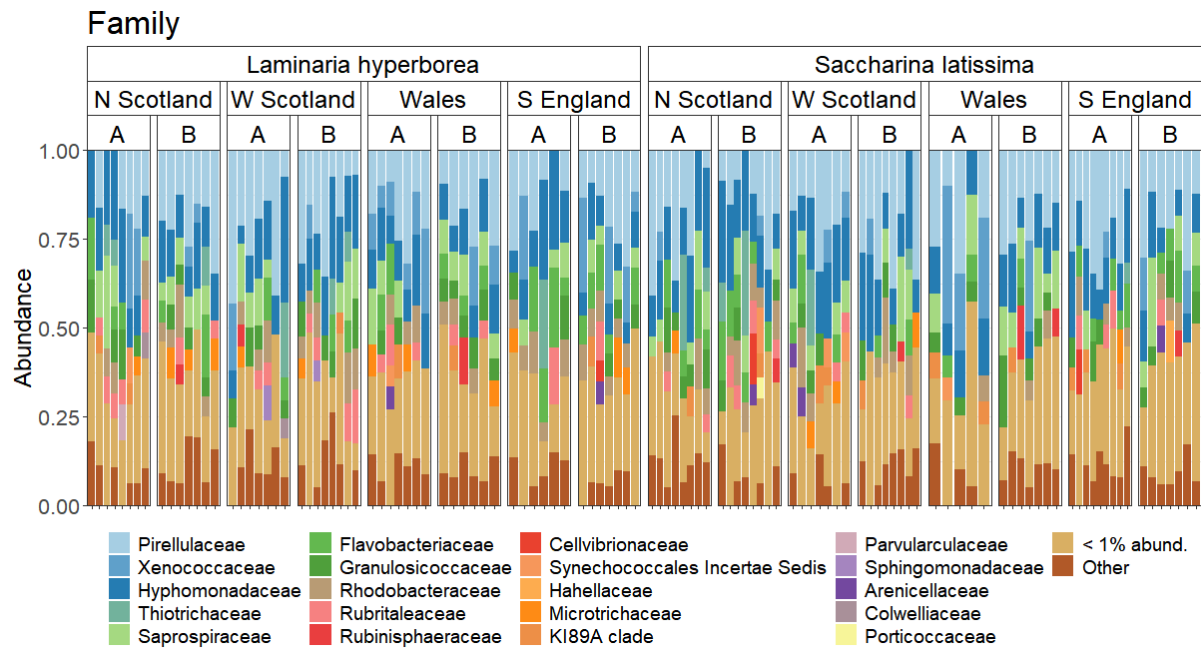
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Figure S1. Rarefaction curves of Amplicon Sequence Variants and sequencing depth of 115 kelp samples from eight sites in the United Kingdom



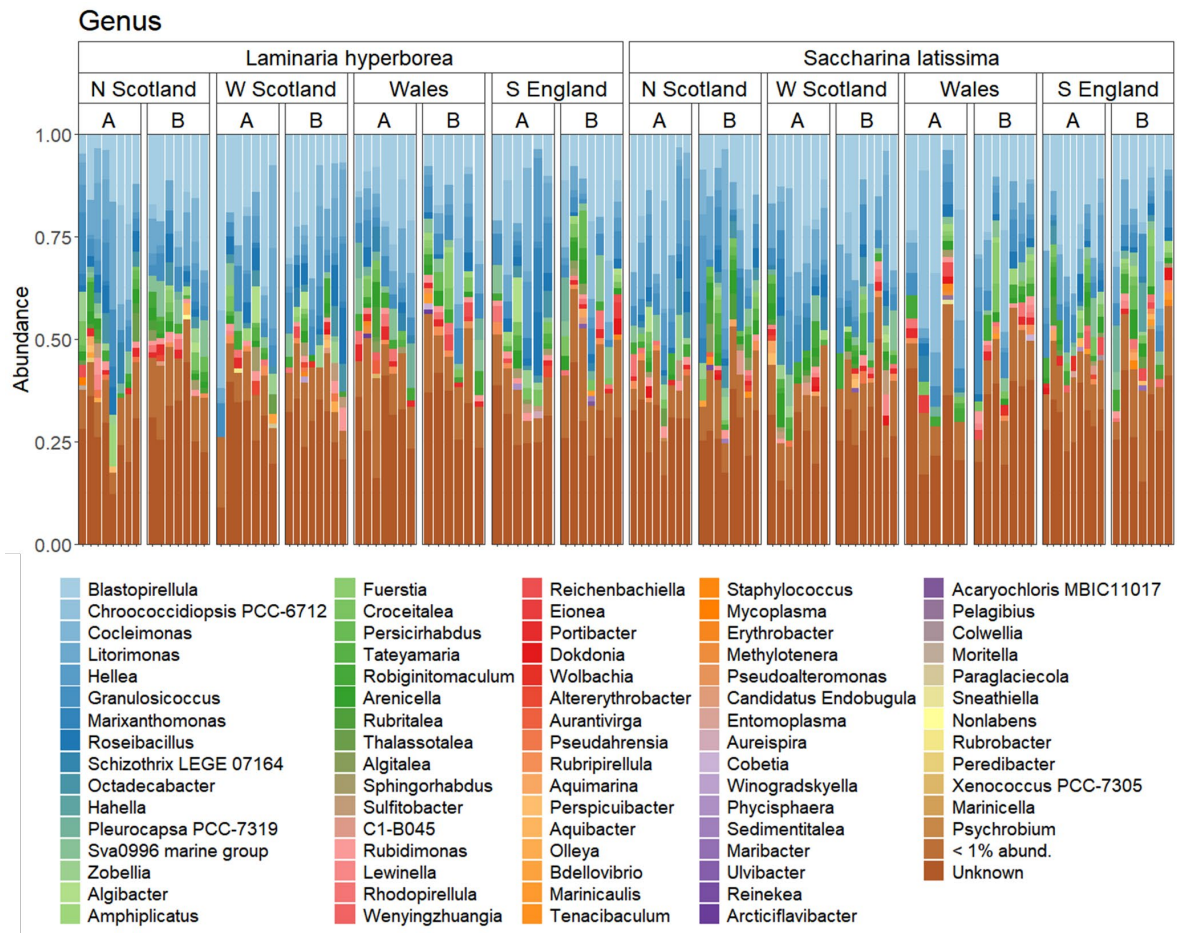
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Figure S2. Relative abundance of bacterial orders in the kelps *Laminaria hyperborea* (left) and *Saccharina latissima* (right) from four study regions (two sites A + B within each region) in the UK. Site locations can be seen in Figure 1. Orders that made a contribution of < 1% to overall study wide abundance were collapsed into a separate category. "Other" represents taxa where underlying taxonomy was not resolved.



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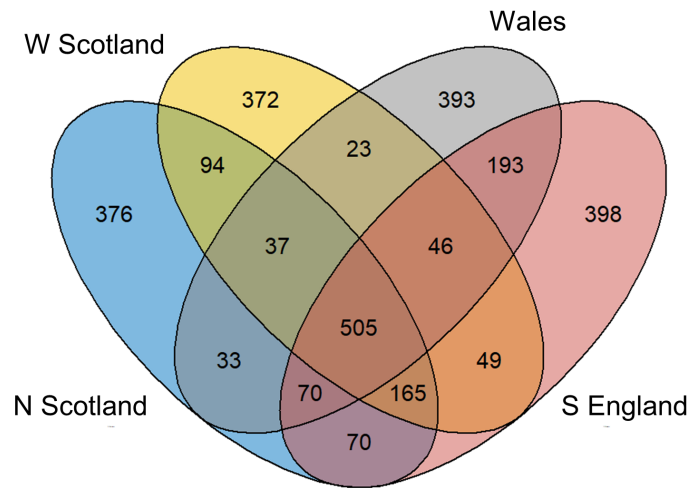
Figure S3. Relative abundance of bacterial Families in the kelps *Laminaria hyperborea* (left) and *Saccharina latissima* (right) from four study regions (two sites A + B within each region) in the UK. Site locations can be seen in Figure 1. Orders that made a contribution of < 1% to overall study wide abundance were collapsed into a separate category. "Other" represents taxa where underlying taxonomy was not resolved.



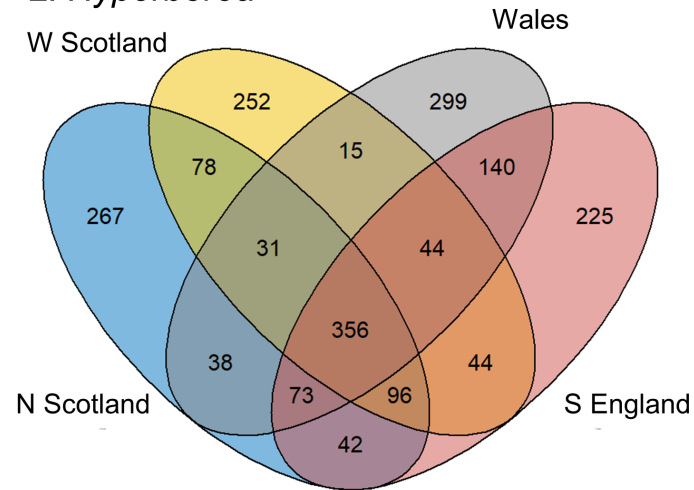
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Figure S4. Relative abundance of bacterial orders in the kelps *Laminaria hyperborea* (left) and *Saccharina latissima* (right) from four study regions (two sites A + B within each region) in the UK. Site locations can be seen in Figure 1. Orders that made a contribution of < 1% to overall study wide abundance were collapsed into a separate category. "Unknown" represents taxa where underlying taxonomy was not resolved.

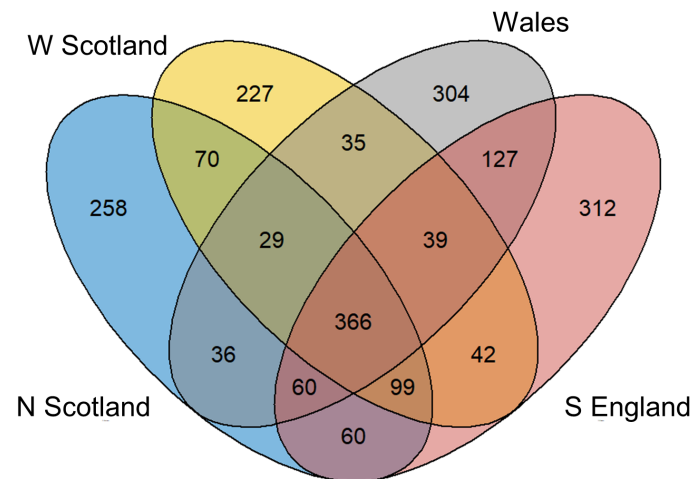
L. Hyperborea + S. latissima



L. Hyperborea



S. latissima



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Figure S5. Venn diagrams showing shared amplicon sequence variants between regions for the kelps *Laminaria hyperborea* and *Saccharina latissima* in the United Kingdom. Locations of study regions are shown in Figure 1.