

## Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors

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1 **Local genomic adaptation of coral reef-associated microbiomes to gradients of**  
2 **natural variability and anthropogenic stressors**

3  
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## 50 **Author Contributions**

51           The manuscript was written by LWK, MY, and FR. Metagenomic analyses were  
52 completed by LWK. Multivariate statistical analyses were completed by GJW. Water samples  
53 for metagenomes and nutrient analysis were collected by KLB, CAC, EAD, AH, CEN, TM,  
54 SAS, ES and FR. The benthic characterizations were completed by JES, GJW, and KLB. YWL  
55 and MH completed all of the library prep and sequencing reactions. RAE provided valuable  
56 computational support. All of the authors offered helpful comments and edits to the manuscript.

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71 **Abstract**

72 Holobionts are species-specific associations between macro- and micro-organisms. On coral  
73 reefs the benthic coverage of coral and algal holobionts varies due to natural and anthropogenic  
74 forcings. Different benthic macroorganisms are predicted to have specific microbiomes. In  
75 contrast, local environmental factors are predicted to select for specific metabolic pathways in  
76 microbes. To reconcile these two predictions, we hypothesized that adaptation of microbiomes to  
77 local conditions is facilitated by horizontal transfer of genes responsible for specific metabolic  
78 capabilities. To test this hypothesis, microbial metagenomes were sequenced from 22 coral reefs  
79 at eleven Line Islands in the central Pacific that together span a wide range of biogeochemical  
80 and anthropogenic influences. Consistent with our hypothesis, the percent cover of major benthic  
81 functional groups significantly correlated with particular microbial taxa. Reefs with higher coral  
82 cover had a "coral microbiome" with higher abundances of *Alphaproteobacteria* such as  
83 *Rhodobacterales* and *Sphingomonadales*, whereas microbiomes of algae-dominated reefs had  
84 higher abundances of *Gammaproteobacteria*, such as *Alteromonadales*, *Pseudomonadales*, and  
85 *Vibrionales*, *Betaproteobacteria* and *Bacteroidetes*. In contrast to taxa, geography was the  
86 strongest predictor of microbial community metabolism. Microbial communities on reefs with  
87 higher nutrient availability (e.g., equatorial upwelling zones) were enriched for genes involved in  
88 nutrient-related metabolisms (e.g., nitrate and nitrite ammonification, Ton/Tol transport, etc.).  
89 On reefs further from the equator, microbes had more genes encoding chlorophyll biosynthesis  
90 and photosystems I/II. These results support the hypothesis that core microbiomes are  
91 determined by the holobiont macroorganisms, and that those core taxa adapt to local conditions  
92 by selecting for advantageous metabolic genes.

93

94 **Statement of significance**

95 Microbial communities associated with coral reefs influence the health and sustenance of the  
96 keystone benthic organisms (e.g., coral holobionts). The present study investigated the  
97 community structure and metabolic potential of microbes inhabiting coral reefs located across an  
98 extensive area in the central Pacific. We found that the taxa present correlated strongly with the  
99 percent coverage of corals and algae, while community metabolic potential correlated best with  
100 geographic location. These finding are inconsistent with prevailing biogeographic models of  
101 microbial diversity (e.g., distance decay) and metabolic potential (i.e., similar functional profiles  
102 regardless of phylogenetic variability). Based on these finding we propose that the primary  
103 carbon sources determine community structure and that local biogeochemistry determines finer  
104 scale metabolic function.

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## 119 **Introduction**

120 Coral reefs are complex ecosystems that provide habitats for diverse, interdependent macro- and  
121 microorganisms. A coral colony itself is a complex holobiont, each made up of a coral polyp and  
122 a suite of prokaryotic microbes, viruses, protists, endolithic fungi and algae, and other  
123 invertebrates (1-4). Some coral-associated microbes confer benefits by, for example,  
124 remineralizing nutrients that are essential for the coral holobiont (5-9). Others contribute to coral  
125 demise by causing a number of specific diseases as well as non-specific detrimental effects (e.g.,  
126 hypoxia) (10-12). On degraded reefs, where coral cover is reduced and the benthic surface is  
127 dominated by fleshy algae, the microbial community includes higher abundances of copiotrophic  
128 microbes, many of which are known pathogens (13). Higher abundances of potential pathogens  
129 on reefs are also known to correlate with higher prevalence of coral disease (14), indicating a  
130 link between the community structure of reef-associated microbes and coral health.

131 Previous studies have described the biogeographic distribution of pelagic microbial communities  
132 by investigating statistical relationships between pelagic microbes and environmental parameters  
133 (15-18). However, application of this approach to coral reef-associated microbes is complicated  
134 by a number of factors. First, for microbial members of specific coral holobionts, microbial  
135 biogeography is directly linked to the distribution of the coral species. Second, reef-associated  
136 microbial communities are influenced by the other benthic macroorganisms present, such as  
137 macroalgae – both calcifying and fleshy, which may vary markedly between locations. Third,  
138 these microbial communities are subject to abiotic factors, such as variable nutrient, temperature  
139 and hydrodynamic regimes associated with a particular geographic location. Given this

140 complexity, understanding the drivers that influence the community structure of reef-associated  
141 microbes requires unraveling numerous interdependent factors.

142 The relationships between microbial community structure, the metabolic capacity of the  
143 assemblage, and their habitat are complex. Numerous taxa share 'core' genes required for  
144 survival in the marine habitat. Supplementing these core housekeeping genes in each strain are a  
145 varied combination of metabolic genes (the pan-genome) associated with specialized pathways  
146 that contribute to fitness under particular local conditions, e.g., limited phosphate availability.  
147 These specialization genes do not respect species boundaries and may be found in multiple taxa  
148 adapted to similar environmental conditions (19, 20). Due to the mobility of these genes via  
149 horizontal gene transfer, the microbes can be considered to share a common gene pool, with  
150 specific genes being enriched within communities in particular niche habitats where they  
151 increase fitness. As a result, the similar community metabolism (i.e., functional redundancy) can  
152 be associated with high phylogenetic variability (21), and likewise communities comprised of  
153 similar taxa may differ in metabolic capabilities (22).

154 The mechanisms that govern community structure and gene flow in complex microbial  
155 communities, such as those associated with benthic marine habitats, remain largely unknown to  
156 the field of microbial ecology. Coral reefs are of particular interest because of their importance  
157 as centers of biodiversity, their contribution to global marine productivity, and their alarming  
158 decline. Coral reefs of the Line Islands (LIs) in the central Pacific offer a unique opportunity to  
159 investigate these questions as they span a latitudinal gradient from 6° north to 11° south. These  
160 islands and atolls (heron referred to as atolls) also span across the Equatorial Counter Current,  
161 Intertropical Convergence Zone and thus experience significant variability in nutrient  
162 concentrations, temperature and precipitation.

163 In addition to oceanographic variability, the northern LIs also span a gradient of human  
164 disturbance where Teraina, Tabuaeran, and Kiritimati support populations of approximately  
165 1,000, 2,500, and 5,000 people, respectively. Reefs at these atolls are impacted by subsistence  
166 and commercial fishing, as well as some pollution (e.g., sewage, chemicals) and agricultural  
167 runoff. Some of the highest known biomass of the fishes for a coral reef ecosystem were  
168 observed on the unpopulated atolls (14, 23), where reefs were characterized by high cover of  
169 reef-building corals and crustose coralline algae, abundant coral recruits, and low levels of coral  
170 disease (14). In contrast, the populated atolls, most notably Kiritimati, had reefs with as low as  
171 2% coral cover and were associated with higher abundances of super heterotrophs, many of  
172 which are known pathogens (13), and higher prevalence of coral diseases (14). Since the reefs at  
173 the uninhabited atolls have been largely spared from such anthropogenic disturbances, they  
174 provide a baseline for a comparative evaluation of the effects of human activity on coral reef-  
175 associated microbes. However, to definitively attribute any observed differences to  
176 anthropogenic activities, the role of other environmental drivers that differ between atolls must  
177 also be examined. For instance, the three inhabited atolls are clustered together in a region  
178 spanning  $<3^\circ$  latitude, inciting a counterargument that local biogeochemical factors were  
179 responsible for reef degradation rather than fishing or other local activities as had been suggested  
180 by a prior study (14).

181 Here we used comparative metagenomics to tease out the key environmental factors driving the  
182 composition and metabolism of reef-associated microbial communities in the LIs. Although the  
183 eleven atolls are clustered in the same oceanic region, they differ in three key environmental  
184 variables that are predicted to influence their microbial communities: nutrient levels, latitudinal  
185 distance from the equator, and the percentage of the benthic surface occupied by various



186 functional groups of macroorganisms. In this study, we collected reef-associated microbes, then  
187 extracted and sequenced the community DNA. Taxonomic and functional annotations were  
188 assigned to the resultant reads by comparison to the SEED protein database. We then quantified  
189 variation in the structure and metabolic potential of the communities in relation to the three key  
190 variables. These comparisons show that (1) the microbial taxa present and their relative  
191 abundances reflect the benthic community whose carbon-containing exudates provide the  
192 primary local energy source, and (2) the presence of various specialized metabolic capabilities  
193 correlates with nutrient levels and other latitude-dependent factors.

194

## 195 **Results**

196 Studies were conducted at 22 reef sites distributed across eleven LIs spanning 18° latitude (Table  
197 S1). At each site, seawater samples were collected at the surface of the benthos for microbial  
198 metagenome preparation and from the immediately overlying water for nutrient analysis. The  
199 macroorganisms comprising the benthic cover were surveyed. Subsequent analyses assessed the  
200 relationships between three predictor variables (benthic macroorganisms, nutrient levels, and  
201 latitude) and both the structure and the metabolic capabilities of the microbial communities at  
202 these atolls.

203 *Nutrient concentration.* Inorganic nitrogen (nitrate+nitrite) and phosphate concentrations were  
204 generally highest near the equator and declined with increasing latitude both north and south  
205 (Figure 1, Table S2). Nitrate+nitrite concentrations ranged from 0.52 to 4.83  $\mu\text{M}$ , whereas  
206 phosphate concentrations varied less (0.15 to 0.44  $\mu\text{M}$ ). When compared to the northernmost

207 (Kingman) and southernmost (Flint) atolls, nitrate+nitrite and phosphate concentrations at  
208 equatorial Jarvis were approximately five- and two-fold higher, respectively.

209 *Benthic macroorganisms*. The benthic cover was quantified as the percentage covered by each of  
210 seven functional groups: hard coral, crustose coralline algae (CCA), calcified macroalgae, soft  
211 coral, fleshy macroalgae, fleshy turf algae, and ‘other’ (Table S2). A list of the genera within  
212 each category is also provided (Table S3). Coral cover varied markedly from 2.2% at one site on  
213 Kiritimati to 86.7% at one site on Malden (mean = 44.4%, Table S2). In general, the uninhabited  
214 atolls were dominated by reef building calcifiers including coral, CCA and calcified macroalgae  
215 (24) while fleshy algae such as turf and fleshy macroalgae dominate the inhabited atolls (14).

216 *Reef-associated microbes*. DNA isolated from microbes sampled at each site was sequenced to  
217 yield 22 metagenomic libraries totaling 2.25 million quality reads (average length 389 bp; Table  
218 S1). The sequenced reads were translated *in silico* into predicted protein sequences; subsequent  
219 comparison to the SEED database provided taxonomic annotations for 21% to 47% of the reads  
220 and assignments to functional subsystems for 27% to 62% of the reads from each site. These  
221 annotations were the basis for comparative analyses of the microbial community structure and  
222 metabolic capabilities across the LI archipelago.

223 The relative abundances of the major taxonomic groups were tabulated (Figure S1), plotted in  
224 2D using non-metric multidimensional scaling (nMDS; Figure 2A), and analyzed for  
225 multivariate structure using SIMPROF (Figure S2). By all measures, geographic location of the  
226 atoll was a poor predictor of similarity for microbial community structure. For example, the two  
227 northernmost atolls, Kingman and Palmyra, clustered with the Southern LIs in Group 1 (Figure  
228 2A) and were most similar to Millennium, one of the southernmost atolls. Likewise Malden and

229 Flint, separated by nearly 900 km, had similar taxonomic composition. In contrast, the metabolic  
230 capabilities (based on level 1 subsystem designations in the SEED; N=20) of microbial  
231 communities in geographic proximity were more similar, forming three groups corresponding to  
232 the northern, middle, and southern atolls (Figure 2B, Figure S3). SIMPROF analyses conducted  
233 at the site level resulted in a higher number of significant groupings, though each site generally  
234 remained located within its own atoll group (Figure S2, Figure S3) provided some exceptions,  
235 particularly in the metabolic groupings (e.g., Flint 2 clustered with Group 3 atolls, Figure S3).  
236 Further analyses were performed to quantify correlations between three key variables and both  
237 microbial community structure and metabolism across the LIs.

238 *Community structure.* The correlations visualized by the CCA (Figure 3A) illustrate that  
239 microbial community structure on LI reefs is closely associated with benthic community  
240 composition. Reefs at all of the uninhabited LIs (Group 1 in Figures 2A and S2) associated with  
241 higher percent cover of reef building calcifiers were characterized by higher abundances of  
242 Cyanobacteria, *Alphaproteobacteria* (i.e., orders *Rhodobacterales* and *Rickettsiales*), and  
243 Firmicutes. Reefs with the highest hard coral coverage, such as Malden and Flint, had higher  
244 abundances of *Sphingomonadales* and Cyanobacteria (Figure 3A). Though the abundance of the  
245 genus *Synechococcus* correlates positively with nutrient concentration in pelagic microbial  
246 communities, here it was positively correlated with the percentage of hard coral cover (Table 1,  $r$   
247 = 0.665,  $p = 0.026$ ). In contrast, hard coral cover showed a strong negative correlation with the  
248 abundance of *Alteromonadales* ( $r = -0.819$ ,  $p = 0.002$ ).

249 The inhabited Group 2 atolls associated with higher percent cover of fleshy macroalgae  
250 (Tabuaeran and Teraina; Figure 3A) had greater abundances of *Gammaproteobacteria* (e.g.,  
251 orders *Enterobacteriales*, and *Pseudomonadales*) and *Betaproteobacteria*. In contrast, the reefs

252 at populated Kiritimati were dominated by fleshy turf algae (58.9-82.4%) and supported a  
253 markedly increased abundance of *Bacteroidetes* (25.1%  $\pm$ 4.2%, N=2) compared to the other  
254 atolls (7.2% $\pm$ 3.5%, N=20). Specifically, five genera within the class *Flavobacteria* (genera  
255 *Croceibacter*, *Dokdonia*, *Gramella*, *Leeuwenhoekiella*, and *Polaribacter*) were consistently  
256 overrepresented compared to sites on other atolls. Overall, the percent coverage of fleshy turf  
257 algae on LI reefs was positively correlated with bacteria from the orders *Flavobacteriales* and  
258 *Alteromonadales* (Table 1,  $r = 0.815$ ,  $p = 0.002$  and  $r = 0.682$ ,  $p = 0.021$ , respectively). The CCA  
259 also depicted a correlation between the percent cover of other benthic organisms and Kiritimati  
260 reefs. Though other benthic organisms contributed to <1% of the benthic composition on most LI  
261 reefs, the 2 sites on Kiritimati had a higher percentage of sand, which contributed to the higher  
262 percent cover of this category (5.2%  $\pm$  0.5%).

263 A distance-based linear model (DistLM) was used to formally quantify which suite of predictor  
264 variables formed the best-fit model (balancing performance with complexity) for explaining  
265 variations in microbial communities across LI reefs. Hard coral alone had the largest impact on  
266 microbial community structure explaining 15.2% of the variation between reefs (Table S4).

267 *Community metabolism.* Distance from the equator was the strongest predictor of community  
268 metabolism, explaining 18.4% of the variation in microbial metabolic potential (Table S4). The  
269 two northern atolls (Group 2 in Figure 2B; Kingman and Palmyra) were characterized by high  
270 abundances of genes encoding cofactors, RNA metabolism, and protein metabolism. Moving  
271 southward, the mid-latitude atolls (Group 3 in Figure 2B; Jarvis, Kiritimati, Teraina, and  
272 Tabuaeran) were characterized by higher abundances of genes for aromatic compound  
273 utilization, iron metabolism, membrane transport, nitrogen metabolism, potassium metabolism,

274 regulation, and virulence. All of the southern Line Islands were combined into one group and  
275 had similar community metabolism (Group 1, Figure 2B).

276 The question remained as to which environmental parameters associated with latitude were  
277 driving these variations. Nutrient levels varied across the LIs as expected due to the influence of  
278 equatorial upwelling (Figure 1). As such, a number of metabolic pathways (SEED level 3  
279 subsystems) demonstrated significant correlations with local phosphate concentrations across all  
280 eleven atolls. These included six pathways positively correlated with phosphate concentration:  
281 conjugative transfer, chemotaxis, nitrate and nitrite ammonification, cobalt-zinc-cadmium  
282 resistance, multidrug resistance efflux pumps, and Ton and Tol transport (Figure 4A, Table S5).  
283 Phosphate concentration was negatively correlated with two metabolic pathways involved in  
284 photosynthesis: chlorophyll biosynthesis and photosystems I and II (Figure 4B and Table S5),  
285 and also with the abundance of *Prochlorococcus* (Table 1). Genes for ribosomal proteins were  
286 also overrepresented at oligotrophic sites (Figure 4B).

287 *Interisland Comparison.* Atolls in close proximity were observed to have similar metabolic  
288 capabilities despite differences in their taxonomic composition. For example, microbial  
289 communities from the geographically close Jarvis and Kiritimati had similar metabolic profiles  
290 (Figure 2), but the taxonomic profile of Jarvis was most similar to Vostok and Starbuck, while  
291 that for Kiritimati was the most dissimilar of all (Figure 2). Conversely, the distant atolls of  
292 Kingman and Malden supported taxonomically similar microbial communities that encoded  
293 divergent metabolic capabilities. Hence, microbial communities composed of different taxa can  
294 encode similar functions, and vice versa.

295

296 **Discussion**

297 This study reports the first large-scale metagenomic survey of the microbial communities  
298 associated with coral reefs that simultaneously characterizes both taxonomic composition and  
299 metabolic capabilities. We have demonstrated that, at the ecosystem level, benthic  
300 macroorganisms most strongly influence the taxonomic composition of the microbial  
301 community, while metabolic specialization genes carried by these taxa vary between locations  
302 and reflect functional adaptations to local oceanographic conditions.

303 For this study, microbial communities were sampled from 22 coral reef sites at eleven atolls  
304 across the Line Island (LI) archipelago, atolls that differed with respect to their benthic  
305 community, nutrient levels, and latitude. The microbes collected by our procedure were closely  
306 associated with the surface of the benthic macroorganisms (corals and algae). As a result, they  
307 included species-specific bacterial components of the coral holobiont (1) as well as specific  
308 bacterial taxa associated with some algal functional groups (1, 25). In addition, the microbial  
309 communities sampled on these reefs reflected selection by the adjacent benthic macroorganisms,  
310 as evidenced by the differences between reef-associated bacterioplankton communities and open  
311 ocean communities (26). There is evidence that reef-associated communities undergo selection in  
312 shallow reef environments by the locally available labile organic matter exuded by the benthic  
313 organisms (27). For example, in an empirical study Nelson and colleagues demonstrated that  
314 exudates collected from coral and macroalgae selectively fostered growth of distinct  
315 bacterioplankton communities (27). Coral exudates promoted communities with higher diversity,  
316 including lineages of *Alphaproteobacteria* with relatively few virulence factors (e.g.,  
317 *Erythrobacteraceae*); whereas exudates from fleshy macroalgae selected for less diverse

318 communities with more copiotrophic *Gammaproteobacteria* lineages (e.g., the families  
319 *Alteromonadaceae*, *Pseudoalteromonadaceae*, and *Vibrionaceae*).

320 *Community structure.* The current study confirms and extends earlier findings (27) by  
321 demonstrating similar correlations between the benthic community composition and the  
322 enrichment of specific microbial taxa on coral reefs *in situ* (Table 1). Consistent with the effects  
323 of individual exudates, high coral cover was associated with higher abundances of  
324 *Alphaproteobacteria*, while the abundant fleshy macroalgae at Tabuaeran and Teraina were  
325 accompanied by more *Gammaproteobacteria* (e.g., *Enterobacteriales* and *Pseudomonadales*). In  
326 addition, the fleshy turf algae that dominated Kiritimati favored *Flavobacteria* (phylum  
327 Bacterioidetes) including genera increased by turf algal exudates (*Dokdonia*, *Gramella*, and  
328 *Leeuwenhoekiella*) (28). Together, these complementary research approaches indicate that coral-  
329 and algae-derived organic exudates enrich for specific types of bacteria living in close  
330 association with coral reefs.

331 Nutrient levels have also been postulated to influence microbial community composition. Here  
332 we tested this hypothesis using the natural nutrient gradient present across the LIs. Due to the  
333 equatorial Pacific upwelling in this region, phosphate and nitrate are elevated at the equator and  
334 decrease with latitude both north and south (Figure 1). In high-nitrate, low-chlorophyll  
335 ecosystems such as this, iron may be the nutrient limiting primary production (29). Other  
336 unspecified biogeographic factors also vary with latitude across the LIs. In this study, neither  
337 nutrients nor other latitude-dependent variables were included in the best fit model for  
338 determining microbial community structure. Therefore, we propose that on these geographically  
339 separate coral reefs, microbial community structure is determined by the available energy source,

340 i.e., the DOC provided in the form of benthic exudates, which provides a mechanism for the  
341 correlations observed between the macro- and microbial components of reef communities.

342 *Community metabolism.* In contrast to community structure, specialized and ecologically-  
343 relevant metabolic capabilities of these communities reflected local nutrient concentrations. For  
344 example, six level 3 metabolic subsystems (the SEED database) correlated positively with  
345 phosphate concentration across the LIs (Figure 4A). Some of these, such as the TonB system,  
346 contribute to nutrient acquisition. The TonB system transports large molecules in through the  
347 outer membrane of Gram-negative Bacteria, e.g., polysaccharides, proteins, and siderophores. Its  
348 importance in marine environments is evidenced by the presence of these genes in marine  
349 bacterial genomes and pelagic metagenomes (30-32), their high levels of expression in  
350 metatranscriptome data (33), and the proteomic identification of their products as the  
351 predominant membrane proteins in pelagic Bacteria (34). In this study, they accounted for nearly  
352 1% of gene function annotations at some high nutrient sites (Figure 4A). Genes of the  
353 conjugative transfer subsystem, also overrepresented at high nutrient sites, may function in  
354 energy and nutrient acquisition via type IV secretion of ectoenzymes and siderophores, and may  
355 support active horizontal gene transfer via conjugation. Conversely, the more oligotrophic sites  
356 exhibited overrepresentation of two photosynthesis pathways (chlorophyll biosynthesis and  
357 photosystems I and II) (Figure 4B and Table S5), as well as greater abundance of  
358 *Prochlorococcus*, a key primary producer in the oligotrophic oceans (Table 1).

359 Previous studies have shown that the anaerobic ammonification of nitrate and nitrite (also  
360 referred to as dissimilatory nitrate reduction to ammonium, DNRA) is significant for nitrogen  
361 metabolism in the diffusive boundary layer, an environment with heterogeneous distribution of  
362 dissolved oxygen during the day (12) that then becomes anoxic at night (35). That anaerobes



363 dominate coral-associated microbial communities suggested that this anaerobic nitrogen  
364 metabolism may be important on coral surfaces (25). An interesting observation from the  
365 nutrient measurements is that atolls with higher nitrate+nitrite availability have lower ammonium  
366 concentrations whereas low nitrate+nitrite atolls have higher ammonium. Nitrate+nitrite to  
367 ammonium ratios were 0.26, 0.29, and 0.22 on Malden, Jarvis, and Kiritimati compared to 3.23  
368 and 1.47 on Flint and Kingman, respectively (Table S2). Therefore, the overrepresentation of  
369 DNRA may reflect the lower abundances of ammonium at these high nutrient sites.

370 Reef-associated microbial communities in high nutrient environments encoded greater metabolic  
371 complexity, suggesting that they carry more specialization genes and thus generally possess  
372 larger genomes (Figure S4). Consistent with this hypothesis, single-copy genes encoding  
373 ribosomal proteins were overrepresented at oligotrophic sites (Figure 4B), indicating that the  
374 community overall possessed smaller genomes compared to those at high nutrient sites.

375 Although both phosphorus and nitrogen concentrations correlated with distance from the equator  
376 ( $r = -0.74$  and  $-0.64$ , respectively, Table S6), neither was as strong a predictor of metabolism as  
377 was latitudinal distance from the equator (as assessed by DistLM analysis). Distance from the  
378 equator may serve as a proxy for other influential but unsampled variables such as seawater  
379 temperature, salinity, PAR, or micronutrient concentrations (e.g., iron). In addition, the limited  
380 sampling (1-4 sites at each atoll) may have obscured significant correlations to specific nutrients.  
381 Had the atoll averages been based on sampling of 20+ sites per atoll, significant correlation with  
382 specific nutrients might have been discernible. Nevertheless, the availability of the  
383 macronutrients nitrate+nitrite and phosphate are posited to be important factors influencing  
384 microbial community metabolism on LI reefs.

385 *Anthropogenic impacts on LI reefs.* The findings of this study indicate that local human  
386 populations influence the reef-associated microbial community indirectly by influencing  
387 composition of benthic macroorganisms. Typically activities such as fishing remove important  
388 grazing herbivore species resulting in increased cover of fleshy algae, and this in turn profoundly  
389 impacts microbial community structure at the populated atolls (Figure 2, Figure S1). Increased  
390 coverage by fleshy algae selects for specific microbes that may be detrimental to coral health  
391 (27, 36), thereby opening additional benthic space for further algal colonization (37).

392 *Discordance between taxa and metabolism.* Both the abundance of specific taxonomic groups  
393 and the community metabolic capabilities of the reef-associated microbial communities varied  
394 across the Line Islands. Both correlated with ecological factors, but did so independent of each  
395 other. As a result, atolls as far apart as Kingman and Malden (~1,400 km) hosted taxonomically  
396 similar communities, but these communities effectuated different metabolisms. Conversely, the  
397 different microbial communities at equatorial Jarvis and Kiritimati encoded similar metabolic  
398 specialization genes. This discordance between taxonomy and metabolism is novel and  
399 intriguing. We hypothesize that while community structure is attributable to the core genes that  
400 classify each taxon, community metabolism reflects the particular complement of specialization  
401 genes that comprise the dynamic genome of each strain present. Previously, strain-specific  
402 adaptation to different nutrient levels had been documented in marine cyanobacteria for genes  
403 involved in phosphate acquisition. The particular genes present and their genomic organization  
404 depended on phosphate availability in each isolate's source environment. Strains of  
405 *Prochlorococcus* that showed 99.9% similarity of their 16S rRNA genes nevertheless possessed  
406 different phosphate metabolism genes located in different genomic locations (19). Conversely,  
407 some more divergent strains that occupied environments with similar nutrient regimes shared

408 similar phosphate gene content and organization. Additionally, although *Prochlorococcus*  
409 typically assimilates only ammonium, in regions of nitrogen limitation strains have adapted to  
410 utilize nitrate and nitrite by using genes acquired horizontally from *Synechococcus* (20).

411 The observed adaptation of microbial community metabolism patterns could have resulted from  
412 either gene acquisition and loss or shifts in the relative abundances of strains adapted to different  
413 conditions. Traditionally, only changes in strain abundance (i.e., beta diversity) have been  
414 considered as possible drivers of rapid adaptation in ecological time. Increased genetic diversity,  
415 i.e., evolution, by mechanisms such as horizontal movement of genes between strains or species,  
416 has been expected to require evolutionary time. We posit that in these microbial communities,  
417 evolution is rapid, occurring in ecological time.

418 Attempts to identify the evolutionary mechanisms active in this situation have been hampered by  
419 the limited representation of marine microbes in databases (38), such as the SEED, due to our  
420 inability to culture most species (39). The availability of single-cell whole genome amplification  
421 methods (40) promises to enable genomic characterization of unculturable marine microbes,  
422 thereby substantially accelerating resolution of this question.

423

## 424 **Materials and Methods**

425 Metagenomic sequence reads were compared to the SEED protein database using BLASTx. For  
426 taxonomic annotation, sequences with significant similarities (E-value  $<10^{-5}$ ) were assigned to  
427 the closest identified microbial representative. For functional annotation, sequences were  
428 assigned the function of the closest identified protein and these functions were then grouped into  
429 metabolic pathways according to the subsystems in the SEED database. Community structure

430 was compared using the relative abundances of 19 higher rank microbial taxa (see SI Text and  
431 Table S7 for clarification of taxonomic groups). Similarly, community metabolism was  
432 determined by comparing the relative abundance of 20 Level 1 subsystem categories in the  
433 SEED database.

434 Non-metric multidimensional scaling (nMDS) analyses were used with the annotated  
435 metagenome data to visualize between-atoll similarity in terms of two discrete response  
436 variables: community structure and community metabolism. For an initial exploration of  
437 potential correlations between the three predictor variables and either microbial community  
438 structure or metabolism, a canonical correspondence analysis (CCA) was performed using the R  
439 package vegan. To formally quantify how much variation in the microbial communities or their  
440 metabolism could be explained by the predictors measured (continuous variables), a  
441 permutational distance-based multivariate linear model (DistLM) was used in PERMANOVA+.  
442 Full methods and any associated references are available in the SI.

443

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459

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## 556 **Figure Legends**

558

559 Figure 1. The Line Islands and their nutrient concentrations. (A) The eleven main atolls sampled  
560 in this study. Scale bar indicates latitude and distance between atolls. Atoll sizes are  
561 proportionate, but not to scale. (B) Average nutrient concentrations at the eleven atolls. Nutrient  
562 concentrations were measured in triplicate for each of the 22 study sites (N = 66) and averaged;  
563 sites were then averaged for each atoll. Solid and dashed error bars show the standard error for  
564 atoll and site replicates, respectively. Average values for each site are provided in Table S2.

565

566 Figure 2. Non-metric multidimensional scaling plots for the relative abundances of taxonomic  
567 similarities (A) and metabolic subsystem similarities (B). Sites were averaged for each atoll. The  
568 2D stress values for are 0.05 and 0.03 for the taxonomic and metabolic similarities, respectively.  
569 Dark gray circles indicate significant groupings from the SIMPROF analysis (Figures S2 and S3;

570 Bray-Curtis similarity, p-value <0.01). Light gray circles cluster atolls with greatest similarity  
571 within each statistically significant group.

572

573 Figure 3. Canonical correspondence analysis (CCA) depicting the correlations between predictor  
574 variables (blue) and the relative abundance of taxonomic similarities (A) and metabolic  
575 similarities (B) at each Line Island. Loading vectors for the taxa and subsystems are shown in  
576 red. Altero, *Alteromonadales*; Betaproteo, *Betaproteobacteria*; Enterob, *Enterobacteriales*;  
577 Oceano, *Oceanospirillales*; OtherAlphas, Other *Alphaproteobacteria*; Pseudomon,  
578 *Pseudomonadales*; Rhodobact, *Rhodobacterales*; Sphing, *Sphingomonadales*; calc macro,  
579 calcified macroalgae; cca, crustose coralline algae; macro, fleshy macroalgae; soft, soft coral;  
580 dist, distance from the equator in degrees latitude.

581

582 Figure 4. Metabolic pathways that correlate positively (A) and negatively (B) with increasing  
583 distance from the equator (decreasing nutrient concentrations) across the Line Islands. Pathways  
584 are level 3 subsystem annotations from the SEED database.