Describing macroecological patterns in microbes: Approaches for comparative analyses of operational taxonomic unit read number distribution with a case study of global oceanic bacteria

Running title: OTU read number distributions in microbes

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Author contributions

RN conceived of the study, analyzed the data, and wrote the first draft; RN, YO, and SM discussed the results and their interpretation and contributed significantly to the final text.

Conflict of interest

The authors declare that they have no conflicts of interest.

Abstract

Describing the variation in commonness and rarity in a community is a fundamental method of evaluating biodiversity. Such patterns have been studied in the context of species abundance distributions (SADs) among macroscopic organisms in numerous communities. Recently, models for analyzing variation in local SAD shapes along environmental gradients have been constructed. The recent development of high-throughput sequencing enables evaluation of commonness and rarity in local communities of microbes using operational taxonomic unit (OTU) read number distributions (ORDs), which are conceptually similar to SADs. However, few studies have explored the variation in local microbial ORD shapes along environmental gradients. Therefore, the similarities and differences between SADs and ORDs are unclear, clouding any universal rules of global biodiversity patterns. We investigated the similarities and differences in ORD shapes vs. SADs, and how well environmental variables explain the variation in ORDs along latitudinal and depth gradients. Herein, we integrate ORDs into recent comparative analysis methods for SAD shape using datasets generated on the Tara Oceans expedition. About 56% of the variance in skewness of ORDs among global oceanic bacterial communities was explained with this method. Moreover, we confirmed that the parameter combination constraints of Weibull distributions were shared by ORDs of bacterial communities and SADs of tree communities, suggesting common long-term limitation processes such as adaptation and community persistence acting on current abundance variation. On the other hand, skewness was significantly greater for bacterial communities than tree communities, and many ecological predictions did not apply to bacterial communities, suggesting differences in the community assembly rules for microbes and macroscopic organisms. Approaches based on ORDs provide opportunities to quantify macroecological patterns of microbes under the same framework as macroscopic organisms.

Keywords: Species abundance distribution, Skewness, Weibull distribution, Whittaker plot

Introduction

Species diversity patterns, which are characterized by numbers of species and individuals, provide great opportunities for understanding the ecological and evolutionary processes that drive global biodiversity (Rabinowitz, 1981; Ricklefs, 2000; Hubbell, 2001, 2013; Loza *et al*., 2017). In general, certain species are dominant while others are rare locally and globally. The processes determining locally common and rare species have been studied in the context of the species abundance distribution (SAD), which is one of the most fundamental methods of describing local diversity (Motomura, 1932; MacArthur, 1960; McGill *et al*., 2007; Doi and Mori, 2013; Ulrich *et al*., 2018b). SAD studies focus on the rarity and commonness of species in a local community, and attempt to reconstruct the background processes that led to those patterns. To date, numerous SAD models have been proposed (reviewed in McGill *et al*., 2007). MacArthur (1957, 1960) developed the broken stick model based on the hypothesized niche portioning process, and Hubbell *et al*. (2001) provided a mechanistic interpretation of observed abundance distributions with well-defined ecological parameters such as dispersal, speciation rate, local abundance, and meta-community size, under the premise of ecological drift. Using these models, we estimated background processes from SAD patterns. Previous SAD investigations have been conducted mainly in plants and animals (e.g., Ulrich *et al*., 2010; Baldridge *et al*., 2016), because large datasets and specific criteria for species delimitation are necessary for SAD. Therefore, the SAD approach has been applied less to studies of microorganisms than those of macroscopic species.

The recent development of molecular techniques, in particular high-throughput sequencing, has made it dramatically easier to capture biodiversity patterns in microbes as well as larger organisms (Lynch and Neufeld, 2015; Schloss *et al*., 2016; Shade *et al*., 2018). High-throughput sequencing technology has revealed that the microbial ecosystem is inhabited by a large number of rare microbial lineages (Fuhrman, 2009),

95 collectively referred to as the "rare biosphere" (Sogin *et al*., 2006; Galand *et al*., 2009;
96 Ser-Giacomi *et al*., 2018; reviewed in Lynch and Neufeld, 2015). The existence of rare
97 lineages strongly impacts community structure and diversity patterns, although their
98 ecological roles and the processes structuring the rare biosphere remain poorly
99 understood (Ser-Giacomi *et al*., 2018). The relationships between operational
100 taxonomic units (OTUs; reviewed in Blaxter *et al*., 2005) and read number have been
101 studied in microbes based on concepts similar to SAD (e.g., Livermore and Jones,
102 2015). Hereafter, we consider OTU read number distributions (ORDs), corresponding
103 conceptually to the rank abundance curve, as being distinct from SADs. OTUs are
104 generally defined as groups based on sequence similarity in maker genes (e.g., SSU
105 rRNA in bacteria and ITS in fungi) (Bálint *et al*., 2016). Numerous previous studies
106 have attempted to identify a general best-fit model for ORD in a particular habitat by
107 comparing various traditional SAD models (e.g., log-series, lognormal, and power-law
108 distributions) (Shade *et al*., 2012, Sherrill-Mix *et al*., 2016, Shoemaker *et al*., 2017,
109 Louca *et al*., 2019). However, few studies have focused on the continuous variation in
110 ORD shape within microbial communities along geographical and environmental
111 gradients (however, see Stegen *et al*., 2016), which has been a major recent trend in
112 SAD studies of macroecological patterns and their assembly processes.
113 In recent years, methods for analyzing macroecological patterns and community
114 assembly by comparing SAD shapes have been developed to overcome the limitations
115 of conventional SAD modeling. Specifically, researchers have recently recognized that
116 multiple processes can generate similar SAD shapes, resulting in the fit of a given SAD
117 model not providing clear evidence to support a particular theory (Mathews *et al*., 2017).
118 An alternative approach to comparing changes in parameter value(s) for a given SAD
119 model or the fits of different SAD models to data from a single site is the collection of
120 abundance data from a variety of sites followed by construction of models to analyze
121 how SAD properties vary with predictor variables (i.e., comparative analyses of SAD

122 shape; White *et al*., 2012, Ulrich *et al*., 2016a, b, Fattorini *et al*., 2016; Borda-de-Água
123 *et al*., 2017, Guerin *et al*., 2017, Mathews *et al*., 2019; reviewed in Mathews *et al*.,
124 2017). Many methods have been developed to evaluate SAD shape. For example, ρ_{norm}
125 has been used to evaluate the relative fits of log-series and lognormal distributions
126 (Ulrich *et al*., 2016 a, b). However, the fits of both log-series and lognormal
127 distributions to community data often weaken with increasing species richness (Ulrich
128 *et al*., 2016a, b, also see Fig. S2), which makes the interpretation of these fits
129 inconsistent along species richness gradients in community data. The gambin
130 distribution (Ugland *et al*., 2007) is often used to evaluate SAD shape (Mathews *et al*.,
131 2014, 2017, 2019), but creation of the gambin distribution requires binning the
132 abundance data into octaves prior to fitting, losing species-level abundance information.
133 In addition, the current criterion used to fit the model is the chi-square test, which
134 generally has a strong dependence on sample size, which makes it difficult to apply the
135 gambin distribution go high-throughput sequencing data with large numbers of both
136 OTUs and reads.
137 The comparative analysis approaches used for SAD shape provide two new insights.
138 First, estimating the constraints of community assembly involves analyzing
139 macroecological patterns. Ulrich *et al*. (2018b) proposed the concept of 'forbidden
140 communities' in SAD shapes, placing limitations on the possible combinations of λ
141 (scale parameter) and η (shape parameter). The parameter λ can therefore be interpreted
142 as a measure of SAD shape-specific evenness. The shape parameter η is associated with
143 an excess of either highly abundant species (low η) or rare species (high η). Specifically,
144 Ulrich *et al*. (2018b) argued that the combination of high η and low λ generates SADs
145 very similar to the well-known broken stick distribution (MacArthur 1957), but those
146 distributions rarely occur in nature. Ulrich *et al*. (2018b) showed that the shape and
147 scale parameters of this distribution have precise ecological interpretations, with the
148 first acting as a measure of the excess of either rare or common species and the second

quantifying the proportion of persistent species in the focal community. In addition, Ulrich *et al*. (2018b) showed that the scale parameter is linearly correlated with failure time mathematically, and that it indicates the relative proportion of adapted species in a community that may be persistent. Thus, these forbidden communities may indicate limited long-term processes such as adaptation and the persistence of the community characterized by the current abundance variation.

Recently, biogeographic and macroecological studies on geographical and climatic variation in SADs have employed large datasets, mainly for plants (White *et al*., 2012; Ulrich *et al*., 2016a, b; Guerin *et al*., 2017). Indeed, large-scale SAD studies have recently gained attention due to the accessibility of databases. A large amount of species abundance data collected across a broad range of environments is available for large-scale comparative SAD research (Fattorini *et al*., 2016; Borda-de-Água *et al*., 2017). For example, Ulrich *et al*. (2016a) showed latitudinal patterns of SAD shape and discussed the processes behind these patterns using a climatic dataset, while Guerin *et al*. (2017) reported that geographical and climatic gradients affect SAD shapes and discussed future changes in their shapes expected as a consequence of climate change, with changes in diversity and ecosystem function.

Here, we integrate recent approaches used for comparative analyses of SAD shape into ORDs. As a case study, we used community datasets generated form the Tara Oceans expedition (Sunagawa *et al*., 2015). Tara Oceans is a project that profiled planktonic and microbial communities in the global ocean using high-throughput sequencing. We investigated the drivers of variation in ORDs from local bacterial communities along a geographic gradient, and assessed the similarities and differences between the geographical patterns of microbes and macroscopic organisms, comparing the ORDs of bacterial communities with those of macroscopic communities. In particular, we studied the similarities and differences in ORD and SAD shapes, with a particular focus on skewness and parameter combinations of the Weibull distribution,

176 and the extent to which environmental variables explain the variation in ORDs along
177 latitudinal and depth gradients. This approach could support further acceleration of
178 microbial studies by revealing the drivers of variation in ORD shape and making
179 comparisons between microbes and macroscopic species.
180

181 **Materials and Methods**

182 *Empirical data*

183 We used 139 oceanic bacterial community datasets generated from the Tara Oceans
184 expedition (Sunagawa *et al*., 2015). Sunagawa *et al*. (2015) extracted merged
185 metagenomic Illumina reads (miTAGs) containing signatures of the 16S rRNA gene
186 (Logares *et al*., 2013), which were mapped to the SILVA SSU rRNA gene sequence
187 database (Quast *et al*., 2013) and clustered into OTUs at the 97% similarity level. The
188 range of total reads per sample was 34,081–184,190, with an average of 90,103.06 ±
189 28,240.38. The OTU count table was summarized at multiple taxonomic levels and can
190 be downloaded from http://ocean-microbiome.embl.de/companion.html (Sunagawa *et*
191 *al*., 2015). We extracted OTUs representing the domain Bacteria for analyses.

192 We calculated the skewness γ of log-transformed relative read numbers to assess the
193 degree of lower curvature (Ulrich *et al*., 2016a), which was compared to a symmetrical
194 lognormal distribution. Negative values of γ indicate an excess of rare OTUs, while
195 positive values represent an excess of abundant OTUs compared to a lognormal
196 distribution that is symmetrical around the mean. Thus, the symmetrical lognormal
197 distribution is not skewed. Asymmetrical lognormal SADs nearly always indicate an
198 excess of rare OTUs, and consequently have negative skewness (McGill, 2003). The
199 log-series model shows an excess of relatively abundant OTUs (associated with positive
200 skewness). We used the parametric function SE (γ) = $(6/n)^{1/2}$ (Tabachnick and Fidell,
201 1996) to test for significant skewness in SADs (Ulrich *et al*., 2016a). In this bacterial
202 community dataset, skewness is positively correlated with the number of rare OTUs

203 (rare biosphere: <0.1 or <0.01, criteria based on Galand *et al*., 2009, Pedrós-Alió *et al*.,
204 2012) (Fig. S1). The relationships among ρ_{norm}, the alpha parameter of the gambin
205 distribution, and skewness are shown in Fig. S3.
206
207 *Comparison between ORDs of microbes and SADs of forest plots*
208 To reveal differences between the ORD shapes of microbes and SAD shapes of
209 macroscopic organisms, we first compared the skewness values of bacterial
210 communities with those of tree communities published in Appendix 1 of Ulrich *et al*.
211 (2016a). In addition, we compared the two-parameter Weibull distribution, which was
212 recently suggested by Ulrich *et al*. (2018b) as a flexible descriptive model for SAD
213 shapes. Ulrich *et al*. (2018b) suggested that the shape and scale parameters of the
214 Weibull distribution have precise ecological interpretations, with the first being a
215 measure of the excess of either rare or common species, and the second quantifying the
216 proportion of persistent species in the focal community. To identify any similar
217 limitations of parameter combinations in bacterial communities, we fitted
218 two-parameter Weibull distributions to datasets of oceanic bacterial communities and
219 compared the ORD shapes for microbes with the empirical results from 534 tree
220 communities worldwide, presented in Ulrich *et al*. (2018a). If the ORDs of bacterial
221 communities show similar constraints, community structuring processes are likely
222 shared between the bacterial and tree communities, although the specific mechanisms
223 are unknown. We used the reduced major axis fitting method, following Ulrich *et al*.
224 (2018b). Values of *fit* < 0.05 indicate an excellent fit, while *fit* > 0.3 is poor. We used
225 the updated stand-alone application RAD 2.0 (Ulrich *et al*., 2010) for fitting of the
226 two-parameter Weibull distributions.
227
228 *Explanatory variables for skewness in ORDs*

229 We propose three hypotheses for determining skewness in microbial communities
230 based on existing studies in macroscopic organisms. First, climatic and geographic
231 factors, which are directly associated with local productivity, drive skewness
232 (productivity hypothesis), with higher productivity facilitating lower skewness
233 (Whittaker, 1975; Hubbell, 1979; Ulrich *et al*., 2016a). Second, local concentrations of
234 nutrients in water negatively affect skewness (nutrient hypothesis), and thus a more
235 eutrophic environment exhibits lower skewness. Last, characteristics of microbial life
236 cycle strategies (i.e., longevity and persistence) negatively influence skewness (r-K
237 strategy hypothesis), with shorter generation times leading to greater skewness. More
238 detailed explanations of those hypotheses and predictions are provided in Table 1. The
239 original dataset from Sunagawa *et al*. (2015) is also included with information about
240 each sample.
241 In the multi-regression model, we set latitude, squared latitude, water depth (m),
242 nitrite concentration (μmol L^{-1}), phosphate concentration (μmol L^{-1}), and minimum
243 potential generation time (h) as explanatory variables for the variation in skewness. The
244 minimum potential generation times of microbial communities, which were determined
245 from codon usage biases (for detailed methods, see Vieira-Silva and Rocha 2010), were
246 used as an index of r-K strategy at the community level. Minimum potential generation
247 time data are also included the original dataset (Sunagawa *et al*., 2015). The data output
248 from high-throughput sequencing generally contains different sequencing depths (i.e.,
249 uneven sampling effort) for each sample. Sampling effort influences observed SAD
250 shape (Preston, 1948). Therefore, to account for the effects of biases in the sampled data,
251 we added the OTU richness and total read number of each sample as covariates in the
252 regression models. To avoid nonlinear effects, we used ln-transformed data for water
253 depth, species richness, and total abundance.
254 The level of collinearity between these explanatory variables was determined by
255 calculating the variance inflation factor (VIF). All variables were standardized to zero

mean and unit variance prior to parameter estimation. We selected explanatory variables according to a threshold VIF value, where VIF > 10 indicates that the model has a collinearity problem (Quinn and Keough, 2002, Neves *et al*., 2015). VIF ranged from 1.10 to 8.26, suggesting a lack of multicollinearity in this multi-regression model. However, the effects of temperature on skewness showed the opposite trends compared to the results of simple correlation (Fig. S4). We excluded temperature from the final analyses, causing the VIFs to range from 1.07 to 3.06. We use the 'car' package of R (Fox *et al*., 2007) to calculate VIFs and the 'rsq' package (Zhang, 2018) to calculate partial r-square coefficients.

Results

The mean of skewness was 0.81±0.17 among 139 samples. All communities showed significant positively skewed trends compared to the symmetric lognormal model (Tabachnick and Fidell, 1996; Ulrich *et al*., 2016a), indicating that all ORD shapes are significantly more similar to log-series distributions than lognormal distributions. Based on the results of t-test, bacterial communities are significantly more skewed than tree communities (Welch's t-test; $p<0.0001$), and thus more similar to the log-series distribution in shape.

Weibull distribution fits to 137 of the 139 communities (98.6%) were moderate (fit < 0.3), while only two (1.4%) were comparatively poor (fit>0.3). The parameter combinations in bacterial communities did not exceed the parameter space for tree communities (Fig. 1), suggesting constraints on ORD shape (Ulrich *et al*., 2018a).

In total, the model explained about 56% of the variance in skewness in 131 ORDs (Table 2). Eight communities lacked some data and were excluded from the analyses of ORD shapes. Skewness increased with increasing minimum potential generation time, and was not associated with productivity, nutrients or r-K strategy, as shown in Table 1.

Discussion

We fit the two-parameter Weibull distribution and clearly showed that the parameters of the bacterial communities were not outside of the limited space described in Ulrich *et al*. (2018b), suggesting some limitation on community assembly processes that is shared between SADs and ORDs. We could explain about 56% of skewness variation in ORDs, revealing the drivers of skewness. Therefore, we conclude that comparative analyses of ORDs shape are also useful for identifying drivers and background processes, and that ORD shapes have similar constraints to those described in Ulrich *et al*. (2018b). These findings emphasize the applicability of comparative analyses of ORD shape in microbial communities. However, the number of case studies using this method remains small, so further empirical studies are needed to fully elucidate ORD patterns and their drivers.

Similarities and differences between SADs and ORDs

Comparison of SADs and ORDs has been suggested as a useful approach to identify general rules across microbial and macroscopic communities (Shade *et al*., 2018). In the present study, we compared SADs of tree communities with ORDs of microbial communities using the two fitted parameters (shape parameter [η] and scale parameter [λ]) of the Weibull distribution. We confirmed similar constraints of parameter combinations in ORDs and SADs of tree communities of $\eta < 3$ and $\lambda < 6$, as originally proposed in Ulrich *et al*. (2018b). These constraints on community structure may indicate limitation due to long-term processes such as adaptation and persistence of the community on variation in abundance (Ulrich *et al*. 2018b). Locey and Lennon (2016) found similar scaling of commonness and rarity across microbes and macroscopic plants and animals, and proposed a universal dominance scaling law that holds true over 30 orders of magnitude. We suggest that the concept of forbidden communities presented by Ulrich *et al*. (2018b) is related to the scaling law (e.g., community persistence is

310 regulated by metabolic rate). Further comparisons of SADs and ORDs may reveal
311 whether similar constraints exist in other taxonomic groups and habitats.
312 We emphasize that ORDs should have similar dataset properties as SADs. We also
313 take a negative view of the common practice of referring to ORDs as SADs and their
314 inclusion in SAD studies. In our analyses, the skewness of bacterial communities was
315 significantly greater than that of tree communities, indicating a larger proportion of rare
316 OTUs among bacteria than among trees. Previous studies in macroscopic organisms
317 have reported that persistent species (i.e., stable communities driven mainly by habitat
318 filtering) exhibit a lognormal SAD (i.e., smaller skewness), while transient species (i.e.,
319 dispersal-driven communities that vary over time) are best modeled with the log-series
320 (i.e., high skewness) distribution (Magurran and Henderson, 2003; Ulrich and Ollik,
321 2004; Ulrich *et al*., 2010, 2016a). Therefore, our results indicate that bacterial
322 communities include larger proportions of transient OTUs if we interpret the results
323 based on findings from macroscopic organisms.
324 There are two major factors that should be taken into consideration to inclusion of
325 ORDs in SAD studies. The first issue is associated with delimitation of species and
326 individuals in microbes (reviewed in Hason *et al*., 2012). Empirically, 97% similarity in
327 (partial) 16S rRNA gene sequences has commonly been used to characterize
328 prokaryotic species-level phylogenetic diversity. However this resolution leads to
329 underestimation of genomic (i.e., species) diversity (Rodriguez-R *et al*., 2018). In tree
330 communities, Hubbell (2013) found differences in SAD shape among taxonomic
331 resolutions (i.e., species, genus, and family) in tropical forest data; specifically, the tail
332 length (rare species) decreased with lower taxonomic resolution. Moreover, researchers
333 generally treat the read number of OTUs as an abundance metric. However, the results
334 of 16S rRNA gene sequencing reveal relative, rather than absolute, abundances of
335 individual lineages. In addition, these results may be biased due to differences in PCR

336 primer specificity and gene copy number among lineages. Thus, care should be taken
337 when comparing those patterns with actual abundance data.
338 The second issue is associated with the community characteristics. In SAD studies,
339 researchers generally focus on a horizontal community composed of a single trophic
340 level, such as "tree community" and "herbivore community" (but see Mathews *et al*.,
341 2019). However, the sequencing data based on a marker gene contains DNA of the
342 entire assemblage in a sample, which makes it impossible to categorize the trophic
343 levels of all microbes and ontogenetic stages. The presence of a persistent microbial
344 seed bank (i.e., *in situ* populations of long-lived rare OTUs) might affect
345 community-level patterns (Gibbsons *et al*., 2013), making it difficult to interpret the
346 relationship between the proportion of rare OTUs and minimum potential generation
347 time. In other words, microbial community datasets include different life stages as one
348 category, analogous to grouping "seed in the soil," "mature tree," and "dead tree" in a
349 dataset of trees. Above, we noted the possibility that bacterial communities include
350 larger proportions of transient OTUs when interpreted based on data for macroscopic
351 organisms. If we consider that long-lived rare OTUs are present *in situ*, the
352 interpretation of a larger proportion of transient OTUs in microbes is directly
353 contradicted. Therefore, it may be difficult to apply ecological rules that were
354 developed in horizontal communities of macroscopic species (Vellend *et al*., 2010,
355 Vellend 2016) to DNA-based microbial datasets, at least those related to the community
356 properties of persistence and transience.
357
358 *Extent to which environmental variables explain variation in ORD shapes in oceanic*
359 *bacteria worldwide*
360 The multi-regression model explained about 56% of skewness variation in ORDs. Using
361 data from oceanic bacterial communities, we confirmed that the patterns predicted from
362 hypotheses based on macroscopic studies were not supported, as shown in Table 1.

363 Furthermore, the skewness was significantly and positively affected by the minimum
364 potential generation time within bacterial communities. This result is in opposition to
365 the prediction based on research in macroscopic organisms (Table 1, 2). To explain the
366 opposing trends in bacterial communities compared to predictions for macroscopic
367 species, we could interpret this result as showing that competitive exclusion is less
368 likely occur in bacteria than in macroscopic communities. In macroscopic organisms,
369 species are adapted to the environment as a community, which makes the relationship
370 between the environment and community structure clear. In animals and plants, when
371 suitable species are present in an environment, unsuitable species are excluded from the
372 community, and the relationship between the environment and community structure is
373 simple. On the other hand, bacteria under unsuitable conditions may continue existing
374 (e.g., enter dormancy), thus appearing in high-throughput sequencing data. Campbell *et*
375 *al*. (2011) compared DNA-based patterns with those based on RNA (i.e.,
376 transcriptionally active OTUs), and suggested that about 12% of amplicon sequences of
377 oceanic bacteria are always inactive.
378
379 **Conclusions and perspectives**
380 Comparative analyses of skewness, ρ_{norm}, and the Weibull and gambin parameters,
381 which are major approaches used recently in SADs, provide researchers a basis for
382 discussing similarities and differences between microbial and macroscopic life. In the
383 near future, comparative approaches between microbes and macroscopic organisms,
384 including environmental DNA (eDNA) metabarcoding studies (e.g., Doi *et al*., 2019),
385 may reveal universal rules determining global biodiversity patterns. Further theoretical
386 frameworks focused specifically on microbes and multi-trophic data are needed, with
387 macroscopic ecology studies such as Hubbell *et al*. (2001) as a starting point for
388 discussion (Rosindell *et al*., 2012). We encourage construction of microbe-specific
389 ecological rules, such as rules explicitly considering the metabolic versatility of

microbes in community assembly processes, as the properties necessary for inclusion in datasets differs from those for macroscopic organisms. At the same time, further comparative analyses may reveal the detailed drivers and facilitate a better understanding of the similarities and differences between the quantitative patterns of macroscopic and microbial communities.

Data Accessibility Statement

All environmental factors, calculated data, and the OTU table used for analyses are presented in Tables S1 and S2, and the original data were published in Sunagawa *et al*. (2015) and http://ocean-microbiome.embl.de/companion.html. The Weibull parameters and skewness of global tree communities published in Ulrich *et al*. (2018a) are available from https://figshare.com/articles/Weibull_fits/5975098 and Appendix 1 of Ulrich *et al*. (2016a), respectively.

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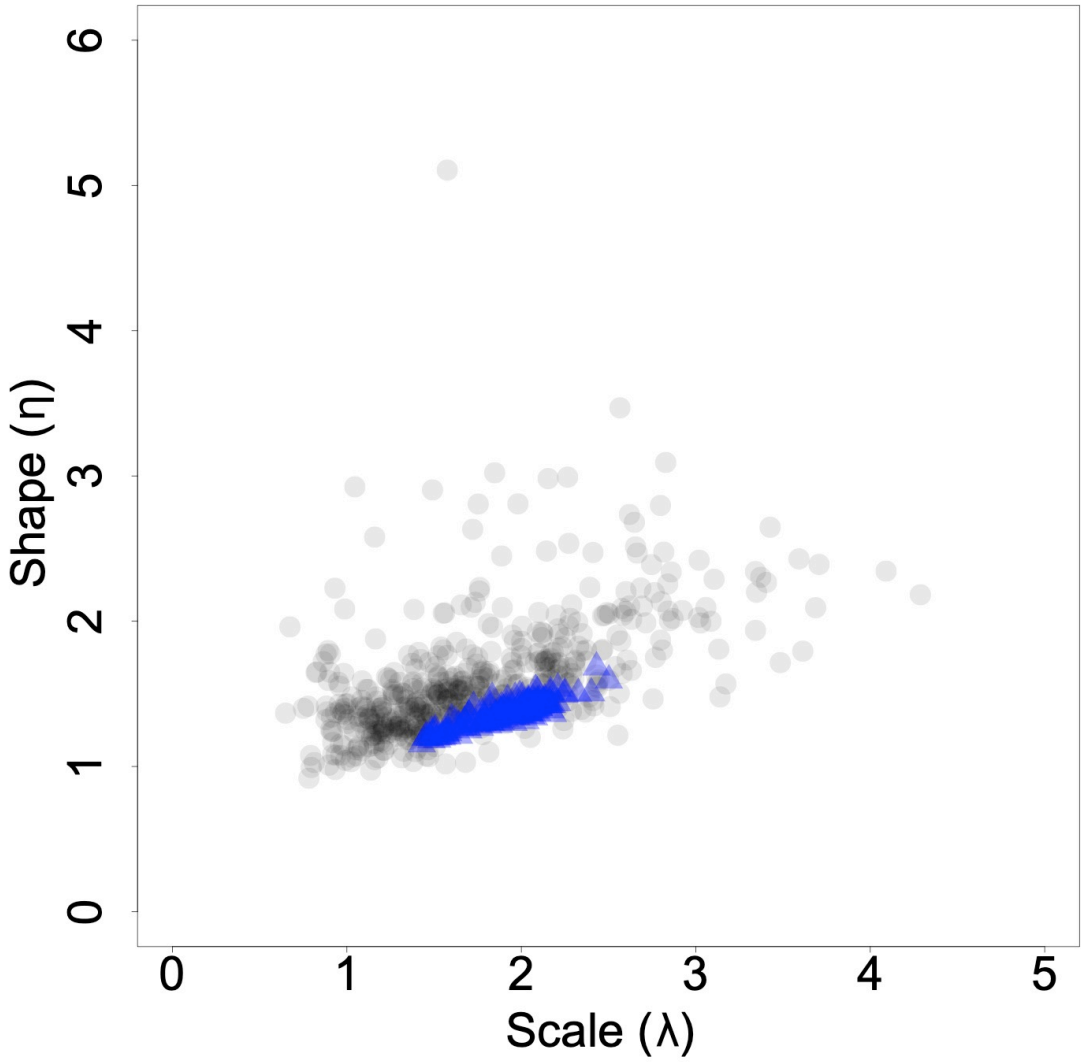
576 **Figure legend**

577 Figure 1 Relationship between shape (η) and scale (λ) parameters. Blue circles indicate
578 the parameters of 139 bacterial communities analyzed in this study. Gray circles
579 indicate the parameters of 534 empirical global tree communities published in Ulrich *et*
580 *al*. (2018a) and https://figshare.com/articles/Weibull_fits/5975098. The parameters of
581 bacterial communities did not exceed the parameter space of tree communities.

582

583 Figure 1



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585 Table 1 Major hypotheses and predictions associated with skewness tested in this study

Hypothesis	Prediction	Related variables	References
(i) Low productivity conditions facilitate skewness (productivity hypothesis).	Skewness decreases toward lower-latitude, deeper, and cooler conditions.	Latitude, Water depth, Temperature	Whittaker (1975), Hubbell (1979), Ulrich *et al*. (2016a)
(ii) Nutrient limitation facilitates skewness (nutrient hypothesis).	Skewness increases toward lower-nutrient conditions.	NO_2, PO_4	Ulrich *et al*. (2016a)
(iii) Dominance of r strategists facilitates skewness (r-K strategy hypothesis).	Skewness decreases with increasing long-lived OTUs.	Minimum potential generation time	Magurran & Henderson (2003), Ulrich & Ollik (2004), Ulrich *et al*. (2016a)

586 **Table 2 Results of the multi-regression model for skewness.**

	β	r^2 *par*	*P*	
Latitude	-0.0425	0.0034	0.5190	
Latitude^2	0.0817	0.0150	0.1740	
ln(Water depth)	0.0304	0.0007	0.7660	
NO_2	0.0530	0.0064	0.3770	
PO_4	0.1397	0.0120	0.1180	
Minimum potential generation time	0.3884	0.1634	<0.0001	***
ln(Total read number)	0.0190	0.0006	0.7830	
ln(OTUs richness)	0.3863	0.1335	<0.0001	***
Whole model		*adj*. R^2	*P*	
		0.5591	<0.0001	***

587 Significance: *<0.05, **<0.01, ***<0.001

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Figure S1 Positive correlations between skewness and rare OTU richness ((a) *adj*. R^2 = 0.3951, p <0.0001, (b) *adj*. R^2 = 0.3966, p <0.0001).

Figure S2 Relationship between lognormal and log-series fits ((a) *adj*. R^2 = –0.0045, p = 0.5387, (b) *adj*. R^2 = 0.1243, p < 0.0001, (c) *adj*. R^2 = 0.0840, p = 0.0003). We followed the methods of Ulrich *et al*. (2016a) for calculating both log-series and lognormal fits.

Figure S3 Comparison among ρ_{norm}, gambin alpha, and skewness. We calculated two indices used for comparative analyses of SAD shapes, ρ_{norm} and gambin alpha. ((a) *adj*. R^2 = 0.3173, p <0.0001, (b) *adj*. R^2 = 0.4440, p <0.0001, (c) *adj*. R^2 = 0.7773, p <0.0001). We followed the methods of Ulrich *et al*. (2016a) and Mathews *et al*. (2014) to calculate ρ_{norm} and gambin alpha, respectively.

Figure S4 Results of simple correlation testing. Significance: *<0.05, **<0.01, ***<0.001, ▪<0.1

Table S1 Extracted bacterial OTU table published in Sunagawa *et al*. 2015.

Table S2 Environmental variables and calculated indices used in this study.

Supplementary text 1 Multimodality of the gambin distribution.