

Resource diversity structures aquatic bacterial communities

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Running Title: Resource Diversity Structures Bacterial Communities

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Key Words: DOM Diversity, Resource Heterogeneity, Bacteria-DOM Interactions, Resource
Specialization, Community Evenness, Microbial Generalists, Ecosystem Metabolomics

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ABSTRACT

24 Microbial diversity is strongly affected by the bottom-up effects of resource availability.
However, because resource pools often exist as heterogeneous mixtures of distinct molecules,
26 resource heterogeneity may also affect community diversity. To test this hypothesis, we surveyed
bacterial communities in lakes that varied in resource concentration. In addition, we
28 characterized resource heterogeneity in these lakes using an ecosystem metabolomics approach.
Overall, resource concentration and resource heterogeneity affected bacterial resource–diversity
30 relationships. We found strong relationships between bacterial alpha-diversity (richness and
evenness) and resource concentration and richness, but richness and evenness responded in
32 different ways. Likewise, we found associations between the composition of the bacterial
community and both resource concentration and composition, but the relationship with resource
34 composition was stronger. Last, in the surveyed communities the presence of resource generalists
may have reduced the effect of resource heterogeneity on community composition. These results
36 have implications for understanding the interactions between bacteria and organic matter and
suggest that changes in organic matter composition may alter the structure and function of
38 bacterial communities.

40

INTRODUCTION

Resource availability is a bottom-up control that has strong effects on the diversity of
42 consumer communities. Theory suggests that resource enrichment promotes diversity and food-
web complexity (Rosenzweig, 1971; Hairston and Hairston, 1993; Abrams and Roth, 1994; Polis
44 and Strong, 1996; Worm *et al.*, 2002), and empirical studies have shown that, in the absence of
top-down control, ecosystems with higher resource concentrations support more diverse and
46 productive communities (Leibold *et al.*, 1997; Leibold, 1999; Hulot *et al.*, 2000; Waldrop *et al.*,
2006). However, the relationship between resources and diversity can be complex (Mittelbach *et*
48 *al.*, 2001; Tilman *et al.*, 1982). For example, while diversity often increases linearly with
resource concentration (Stevens and Carson, 2002), it can also exhibit more complex, non-linear
50 relationships where diversity peaks at intermediate concentrations (Leibold, 1999). Such
responses have been attributed to a range of processes including variation in competitive ability
52 among consumers (Leibold, 1999), shared limitations across species (Stevens and Carson, 2002),
and trophic interactions (Holt *et al.*, 1994; Carpenter *et al.*, 2001).

54 Another feature that may influence resource–diversity relationships is the heterogeneity
of the resource pool. Resources are often considered as homogenous pools, but many resources
56 exist as heterogeneous mixtures of multiple forms (Ashton *et al.*, 2010; Schoener, 1974; Turner,
2008). Resource heterogeneity has the potential to promote consumer diversity via niche
58 partitioning (Schoener, 1974; Finke and Snyder, 2008). For example, plants have been shown to
partition different forms of nitrogen (e.g., NH₄, NO₃, organic N) in ways that may promote
60 species coexistence (McKane *et al.*, 2002; Schimel and Bennett, 2004; Andersen and Turner,
2013). Likewise, different phosphorus resources (e.g., phosphate vs. phytic acid) can alter the
62 diversity and function of aquatic bacterial communities (Muscarella *et al.*, 2014), taxa in

microbial biocrusts have non-overlapping resource preferences (Baran *et al.*, 2015), and
64 phytoplankton are capable of partitioning the light spectrum in ways that allow for species
coexistence (Stomp *et al.*, 2004).

66 The effects of resource heterogeneity may depend on the degree to which communities
are comprised of generalist or specialists. If communities are made up primarily of resource
68 generalists, then the total concentration of a resource should have a stronger influence on
diversity because species do not differ in their response to different resources (Stevens and
70 Carson, 2002). In contrast, if communities are made up of resource specialists, then resource
heterogeneity may promote consumer diversity by providing unique resource niches for
72 consumers to partition (Glasser, 1984; Levine and HilleRisLambers, 2009). Together, resource
heterogeneity and resource acquisition strategy (i.e., generalists versus specialist) may help
74 resolve unexplained variation in resource–diversity relationships.

For heterotrophic organisms, an important resource used for growth and physiological
76 maintenance is organic matter. Organic matter is heterogeneous and consists of molecules that
differ in chemical structure, origin, and age (Stevenson, 1994). In aquatic ecosystems, dissolved
78 organic matter (DOM) is often classified based on origin (autochthonous vs. allochthonous) and
bioavailability (labile vs. recalcitrant). DOM can also be characterized based on its optical
80 properties (Fellman *et al.*, 2009; Weishaar *et al.*, 2003) and functional groups (e.g., humic acids)
(Croué, 2004). However, these characterizations may not adequately describe DOM composition
82 because other chemical features, including molecular weight, oxidation state, stoichiometry, and
chemical structure, can influence the metabolism of organisms that consume DOM, (Cory and
84 McKnight, 2005; Cherif and Loreau, 2007; Lennon and Pfaff, 2005; Berggren *et al.*, 2010).
However, recent technological advances have made it possible to more thoroughly characterize

86 DOM diversity at the molecular level (Moran *et al.*, 2016; Petras *et al.*, 2017; Broeckling *et al.*,
2008). Therefore, there is now the opportunity to understand the linkages between DOM
88 heterogeneity and consumer diversity and characterize resource-diversity relationships (Töpper
et al., 2012; Alonso-Sáez and Gasol, 2007; Gómez-Consarnau *et al.*, 2012; Osterholz *et al.*,
90 2018).

In this study, we measured aquatic microbial communities and DOM chemistry to
92 understand how resource heterogeneity contributes to resource–diversity relationships. We
measured bulk resource concentration measurements and used high-resolution mass
94 spectrometry to quantify resource heterogeneity. We also characterized aquatic bacterial
community diversity using 16S rRNA sequencing. Furthermore, we used species-resource co-
96 occurrence to test the hypothesis that communities dominated by specialists would respond
stronger to resource heterogeneity than to resource availability. Our results support the view that
98 resource heterogeneity promotes bacterial community diversity, but the contribution of DOM
resource heterogeneity may be dampened when DOM generalists dominate bacterial
100 communities.

102 **METHODS**

Study System and Sampling — The Huron Mountains nature preserve is a 5300 ha tract of
104 private land in the upper peninsula of Michigan, USA. The area is part of the Superior Bedrock
Uplands region (Schaeztl *et al.*, 2013). The surrounding forests are primarily old-growth
106 hemlock-northern hardwoods (Woods, 2000), and the inland water bodies are part of the Pine
River Watershed, which drains into Lake Superior. Using a van Dorn sampler, we obtained
108 surface water samples (0.5 m) from 10 lakes in the Huron Mountains during July 2011 (Fig. S1,

Table 1). In addition, we measured dissolved oxygen concentrations, temperature, pH, and
110 conductivity at the time of sampling using Quanta Hydrolab water quality sonde, and we
measured chlorophyll *a* concentration in the lab after cold ethanol extraction of 0.7 μm -filtered
112 (Whatman GF/F) water samples using a Turner Biosystems Fluorometer (Table 1).

114 **Resource Concentrations** — With the water samples, we measured the concentrations of
dissolved organic carbon (DOC), total nitrogen (TN) and total phosphorus (TP). We measured
116 DOC concentrations by oxidation and non-dispersive infrared detection on 0.7 μm -filtered
(Whatman, GF/F) samples using a Shimadzu TOC-V carbon analyzer. We measured TN on
118 unfiltered samples using a Lachat FIA 8500 auto-analyzer (Hach, Loveland CO) after
ammonium peroxydisulfate/sulfuric acid digestion (Lachat, 2005). We measured TP on
120 unfiltered samples spectrophotometrically using the ammonium molybdate method and oxidation
by persulfate digestion (Wetzel and Likens, 2000).

122
Resource Heterogeneity — To estimate resource heterogeneity, we characterized the
124 composition of dissolved organic matter (DOM) for each lake using ecosystem metabolomics.
We extracted DOM from each sample using solid phase extraction (SPE) (Dittmar *et al.*, 2008).
126 Briefly, we acidified 1 L of 0.7 μm -filtered (Whatman, GF/F) water to pH 3.0 with 4N HCl. We
then passed the water sample through an SPE cartridge (Discovery-18, Supelco, Bellefonte PA)
128 at a flow rate $\leq 5 \text{ mL min}^{-1}$ using vacuum pressure. Columns were pre-conditioned using 6 mL
100% methanol followed by 6 mL pH 3.0 ultra-pure H_2O . We filtered the sample until no sample
130 remained or until the cartridge became clogged (recording the final volume filtered) and dried
the filter with N_2 gas for 5 minutes. We eluted the DOM from the column using 100 % methanol

132 and evaporated the methanol at 25 °C using vacuum centrifugation. A consistent amount of
purified DOM was then separated on Waters Acquity ultra-performance liquid chromatography
134 T3 column (1.8 μ M, 1.0 x 100 mm) using water with a 0.1% formic acid-acetonitrile gradient
and analyzed using negative electrospray ionization with quadrupole time of flight mass
136 spectrometry (Q-TOF MS; Waters G2 Q-TOF) and indiscriminate tandem MS (idMS/MS) at the
Colorado State University Proteomics and Metabolomics Facility. Q-TOF MS provides high
138 resolution, accurate mass quantification and idMS/MS provides high collision energy
fragmentation without precursor ion selection acquired concurrently with low-collision energy
140 MS data. For each sample, raw data files were converted to .cdf format, and a matrix of
molecular features as defined by retention time and ion mass (m/z) was generated using the
142 XCMS package in R (Smith *et al.*, 2016) for feature detection and alignment. Raw peak areas
were normalized to total ion signal, and the mean area of the chromatographic peak was
144 calculated from duplicate injections. Features were grouped based on an in-house clustering tool,
RAMClustR, which groups features into spectra based co-elution and covariance across the full
146 dataset, whereby spectra are used to determine the identity of observed compounds in the
experiment (Broeckling *et al.*, 2014). We used field-prepared ultrapure water as controls and
148 subtracted control peaks from sample peak heights. We multiplied control peaks by 1.1 to
provide conservative blank subtraction. A subset of the clustered dataset was referenced to the
150 NISTv14 tandem (MS/MS) library, which contains 193,119 spectra of 43,912 precursor ions
from 8,531 chemical compounds, and also screened for matches in Metlin for putative compound
152 identification. Retention time was used as a proxy for polarity, and the heaviest ion (m/z) in the
clustered spectra was used as a proxy for molecular weight. We recognize that the heaviest ion
154 may not be representative of the molecular weight in all cases due to, for example, the formation

of dimers and potential in-source fragmentation, however, electrospray is the gentlest type of
156 ionization and is often related to the mass of the analytes. We define “DOM components” as the
chemical features identified in DOM samples by ecosystem metabolomics.

158

Microbial Composition — We used an RNA based approach to characterize bacterial
160 community composition by sequencing the 16S rRNA gene transcript. We extracted total nucleic
acids using the MoBio Power Water RNA extraction kit (Carlsbad, CA). Nucleic acid extracts
162 were cleaned via ethanol precipitation and RNA extracts were treated with DNase I (Invitrogen)
to degrade residual DNA. We synthesized cDNA via the SuperScript III First Strand Synthesis
164 Kit using random hexamer primers (Invitrogen). Once cDNA samples were cleaned and
quantified, we amplified the 16S rRNA gene transcript (cDNA) using barcoded primers (515F
166 and 806R) designed to work with the Illumina MiSeq platform (Caporaso *et al.*, 2012). We
purified the sequence libraries using the AMPure XP purification kit, quantified using the
168 QuantIt PicoGreen kit (Invitrogen), and pooled libraries at equal molar ratios (final
concentration: 20 ng per). After pooling, we sequenced the libraries on the Illumina MiSeq
170 platform using 250 x 250 bp paired end reads (Illumina Reagent Kit v2) at the Indiana University
Center for Genomics and Bioinformatics Sequencing Facility. Paired-end raw 16S rRNA
172 sequence reads were assembled into contigs and filtered based on quality score, length, and
ambiguous base calls. After filtering, we aligned our sequences to the Silva Database (version
174 123). Chimeric sequences were detected and removed using the VSEARCH algorithm (Rognes
et al., 2016). We then created operational taxonomic units OTUs by first splitting the sequences
176 based on taxonomic class (using the RDP taxonomy) and the binning sequences in OTUs based

on 97% sequence similarity. All initial sequence processing was completed using the software
178 package *mothur* (version 1.40.5; Schloss *et al.*, 2009).

180 **Resource Heterogeneity and Community Diversity** — First, we tested the hypothesis that
resource heterogeneity affects bacterial community alpha diversity. We used linear models to
182 determine if higher resource concentrations or more types of DOM resources (i.e., resource
richness) would affect the richness and evenness of bacterial communities. We transformed
184 (Box-Cox), centered, and scaled (i.e., divided by standard deviation) resource concentration and
species richness to meet model assumptions of equal variance and normality (Neter *et al.*, 1996).
186 We subsampled bacterial communities using rarefaction to correct for differences in sample size
due to sequencing depth (Hughes and Hellmann, 2005; James and Rathbun, 1981). We rarefied
188 communities and calculated species richness as the number of OTUs observed and species
evenness using Simpson's evenness (Smith and Wilson, 1996). We used the Box-Cox-
190 transformed DOC concentration as the measure of resource concentration and we calculated
resource richness as the number of distinct DOM peaks observed in each sample.

192 Next, we tested the hypothesis that resource heterogeneity affects community beta
diversity by comparing resource concentrations and DOM composition to bacterial community
194 composition. We used distance-based redundancy analysis (dbRDA; Legendre and Anderson,
1999) to test for relationships between: 1) resource concentration and community composition
196 and 2) resource composition and community composition. dbRDA is a multivariate linear model
technique that uses quantitative factors explaining differences in multivariate community
198 composition data. We used the Box-Cox-transformed DOC concentration as the measure of
resource concentration. To use DOM composition as a predictor in our dbRDA model, we used

200 principal coordinates analysis (PCoA), based on relative abundances and Bray-Curtis
dissimilarity, to decompose DOM composition into orthogonal linear components (Legendre and
202 Legendre, 2012). To represent the DOM composition, we used the DOM PCoA axis scores for
each sample. As the response in the dbRDA model, we relativized OTU abundances and used
204 Bray-Curtis distances to compare community composition across samples. Significance tests of
our dbRDA model were conducted based on 10,000 permutations. All calculations were done in
206 the R statistical environment (R Core Team, 2012) using the ‘vegan’ package (Oksanen *et al.*,
2013).

208

Consumer-Resource Specialization — To test the hypothesis that the response to resource
210 heterogeneity depends on whether communities were dominated by generalists or specialists, we
used consumer-resource co-occurrence to define generalists and specialists. We defined resource
212 generalists and specialists based on co-occurrence analysis, which was performed using
Spearman’s rank correlations between DOM components and bacterial OTUs. We used the
214 relative abundances of DOM components and the relative transcript abundances of bacterial
OTUs. We inferred interactions based on correlations with coefficients $> |0.7|$ (Williams *et al.*,
216 2014), and we tested for significance using a permutation test based on randomizations with the
independent-swap algorithm (Gotelli, 2000). We defined resource generalists as those taxa with
218 four or more significant negative resource interactions. We used the negative interaction as a
proxy for potential resource consumption. To understand the spatial extent of individual taxa, we
220 defined cosmopolitan taxa as those found in $\geq 90\%$ of the sampled lakes and we determined
how many resource generalists were also cosmopolitan taxa. All calculations were done in the R
222 statistical environment.

RESULTS

224 **Resource Composition and Heterogeneity** — The lakes sampled captured a range of bulk
resource concentrations (Table 1), and many bulk resource concentrations were correlated. For
226 example, the concentrations of dissolved organic carbon (DOC) and total nitrogen (TN) were
highly correlated ($\rho = 0.97$, $p < 0.001$, Fig. S2). Using ecosystem metabolomics, we
228 characterized the dissolved organic matter (DOM) pool and detected 712 compounds across the
sites. We refer to these molecules as DOM components. Based on the relative abundances of
230 DOM components, sites were on average 37 % dissimilar in DOM composition. Using principal
coordinates analysis (PCoA), we could explain 71 % of the variation in DOM composition
232 across sites using three dimensions (Fig. 1). The variation in DOM composition was significantly
related to DOC ($r^2 = 0.68$, $p = 0.01$), TN ($r^2 = 0.70$, $p = 0.01$), Chl a ($r^2 = 0.69$, $p = 0.02$), and pH
234 ($r^2 = 0.58$, $p = 0.03$), but there were no significant relationships with TP ($r^2 = 0.27$, $p = 0.34$) or
surface area ($r^2 = 0.30$, $p = 0.26$). In addition, we found a negative relationship between the
236 richness of DOM components and the concentration of DOC ($p < 0.01$). We used DOC to
represent resource concentration and the DOM PCoA scores to represent DOM composition in
238 further analyses. We identified influential DOM components as those correlated ($\rho > |0.70|$)
with variation in the DOM PCoA axes (Fig. S3). We identified 172 influential DOM
240 components.

242 **Community Composition and Resource-Diversity Relationships** — Across the 10 lakes, we
identified 5,085 bacterial operational taxonomic units (OTUs) based on 16S rRNA transcript
244 sequencing. When rarified, lakes varied in taxonomic richness and evenness (Fig. 2). Using

Bray-Curtis distances and relative transcript abundances, lakes were on average 62 % dissimilar
246 to one another based on bacterial community composition.

First, we tested for relationships between resources and bacterial alpha-diversity. We
248 used linear regression to test for resource-diversity relationships between bacterial community
diversity (richness and evenness) and both resource concentration and DOM richness. As
250 predicted, bacterial alpha-diversity was affected by resource concentration (Fig. 2). OTU
richness was positively related to resource concentration ($r^2 = 0.66$, $p = 0.008$) but
252 negatively related to DOM richness ($r^2 = 0.50$, $p = 0.023$). In contrast, OTU evenness was
positively related to DOM richness ($r^2 = 0.67$, $p = 0.003$) but negatively related to resource
254 concentration ($r^2 = 0.49$, $p = 0.022$).

Next, we tested for relationships between resources (concentrations and composition) and
256 bacterial beta-diversity using distance-based redundancy analysis (dbRDA). Based on the
dbRDA models, resource concentrations explained 28 % of the variation in bacterial community
258 composition ($p = 0.002$), and DOM composition explained 45 % of the variation in bacterial
community composition ($p = 0.03$, Fig. 3). However, when we partitioned the explained
260 variation among the DOM PCoA axes, only DOM Axis 2 was significant ($r^2 = 0.70$, $p = 0.017$).
In addition, this DOM axis was correlated to variation along OTU PCoA Axis 1 ($\rho = 0.83$,
262 $p = 0.002$; Fig. 3). Last, we tested for relationships between resource concentration and DOM
composition. We found a significant correlation between resource concentration and DOM Axis
264 2 ($\rho = 0.69$, $p = 0.03$).

266 **Consumer–Resource Specialization** — Based on consumer-resource co-occurrence analysis
and spatial occurrence, we classified generalist and cosmopolitan bacteria. We found that 1.3%

268 of taxa (68 OTUs) were resource generalists, and 4.5 % (233 OTUs) were cosmopolitan taxa. Of
the resource generalists, 74 % were also found to be cosmopolitan taxa. Proportionally, resource
270 generalists and cosmopolitan taxa were substantial across all lakes (Fig. 4). For both groups,
there was a significant negative relationship between relative abundance and resource
272 concentration (Fig. 4). In addition, the proportion of resource generalists was related to DOM
composition based on DOM Axis 2 ($\rho = 0.81$, $p = 0.004$), but not DOM Axis 1 ($\rho = 0.08$,
274 $p = 0.82$). Taxonomically, both resource generalists and cosmopolitan taxa were diverse. For the
resource generalists, the majority belonged to the classes Alphaproteobacteria (14) and
276 Planctomycetacia (11), but Verrucomicrobiae (8) and Actinobacteria (7) were also common. At
the family level, the resource generalists represented groups including Acetobacteraceae,
278 Caulobacteraceae, Planctomycetaceae, Sphinomonadaceae, and Verrucomicrobiaceae. For the
cosmopolitan taxa, the majority belonged to the classes Alphaproteobacteria (58) and
280 Betaproteobacteria (50), but Gammaproteobacteria (14), Actinobacteria (21), Planctomycetacia
(17), and Sphingobacteria (12) were also common. At the family level, the cosmopolitan taxa
282 represent groups including Acetobacteraceaea, Alcaligenaceae, Bulkholderiaceae,
Caulobacteraceae, Chitinophagaceae, Comomonadaceae, Flavobacteriaceae, Planctomycetaceae,
284 Rhodobacteraceae, Spartobacteria, Sphinomonadaceae, and Verrucomicrobiaceae.

286

DISCUSSION

288 We found evidence that resource concentration and resource heterogeneity affect
bacterial resource–diversity relationships. Our data suggest that there is a significant relationship
290 between resources (concentration and richness) and bacterial community alpha diversity.

Likewise, resource concentration and composition explained variation in bacterial community
292 composition (beta-diversity), although to differing degrees. Last, DOM generalists were
prevalent in the surveyed microbial communities and that there was a negative relationship
294 between the proportion of generalists and the concentration and composition of DOM. Together,
our results suggest that DOM resource heterogeneity affects aquatic microbial communities, and
296 that DOM resources may influence aspects of community diversity (e.g., species evenness) and
community composition. However, when generalists dominate communities, the effects may be
298 limited potentially due to complex food-web interactions. Based on our findings, we argue that
organic matter composition plays an important role in structuring aquatic microbial communities,
300 and that changes in organic matter composition owing to land use modifications and changing
terrestrial plant communities may alter the structure and function of aquatic bacterial
302 communities.

304 **Resource Heterogeneity in Microbial Food Webs**

Resource heterogeneity affected the diversity of aquatic bacterial communities. We found that
306 DOM resources were heterogeneous across lakes — on average lakes were 37 % dissimilar in
their DOM composition. As such, resource heterogeneity may help explain the variation in
308 resource-diversity relationships along resource concentration gradients. We tested this hypothesis
and found that while resource concentration explained 28 % of the variation, DOM resource
310 composition explained 45 % of the variation in bacterial community composition across lakes.
These findings suggest that different types of bacteria use and potentially specialize on different
312 types of resources, which has been observed elsewhere. For example, it has been shown that
some bacteria primarily use algal-derived resources (Sarmiento and Gasol, 2012; Jaspers and

314 Overmann, 2004) while others primarily use terrestrial-derived resources (Guillemette *et al.*,
2015; Roehm *et al.*, 2009). Therefore, lakes receiving different resource inputs may be expected
316 to contain different bacterial communities. Thus, DOM resource heterogeneity is a potential
mechanism to explain the diversity within and between bacterial communities.

318 Resource diversity (i.e., DOM richness) was positively correlated with OTU evenness,
but negatively correlated with OTU richness (Fig. 2). Resource diversity is likely to influence
320 OTU evenness because evenness, a measure of equitability among taxa, may reflect the
frequency of species traits (Hillebrand *et al.*, 2008; Hill, 1973), such as enzymes needed to
322 uptake and metabolize different DOM components. Furthermore, changes in evenness have been
linked to altered species-interactions, coexistence, and ecosystem functions (Hillebrand *et al.*,
324 2008). If resources represented niches to be partitioned, resource diversity should promote
species diversity because resource diversity provides unique niches to species to partition
326 (Werner, 1977; Glasser, 1984; Schoener, 1974). Because we observed an increase in evenness
but not in richness with greater resource diversity, our findings suggest that the increased
328 evenness observed in communities represents changes in abundances but not the addition of new
taxa. Furthermore, the change in evenness — an increase — suggests that the changes in
330 abundance benefit intermediate rank taxa. Together, our results support the hypothesis that
resource heterogeneity contributes to observed resource-diversity relationships. In addition, we
332 propose that DOM resource heterogeneity may promote more diverse communities by increasing
species equitability and benefiting taxa that comprise the middle ranks of the bacterial
334 community – “The Microbial Middle Class”.

336

Resource Substitutability

338 One possible explanation for why resource heterogeneity may only have weak effects in some
habitats is that many resources are substitutable. Two resources are substitutable when either can
340 each be used for growth and reproduction while the other is absent (Tilman, 1980). For example,
some plants are able to grow using ammonium, nitrate, or even organic nitrogen as a source of
342 nitrogen (Haynes and Goh, 1978; McKane *et al.*, 2002; Schimel and Bennett, 2004), and
zooplankton such as *Daphnia* can use algae, cyanobacteria, and bacteria independently as food
344 sources (Demott, 1998). Likewise, aquatic ecosystems contain numerous phosphorus resources
but some have similar effects on the structure and function of aquatic microbial communities
346 (Muscarella *et al.*, 2014).

In this study, we found numerous DOM components that appear to have similar
348 consumer-resource co-occurrence patterns (Fig. S4). One explanation is that many DOM
components are substitutable. At a chemical level, resources with the same core molecule can be
350 substitutable. For example, vanillate and ferulate share an internal benzene structure and are used
by the same metabolic pathways (Buchan *et al.*, 2000). In addition, extracellular enzymes often
352 degrade aliphatic polymers of different lengths into identical monomers (Rojo, 2009). As such,
many DOM components are likely substitutable. We used chemical databases to identify the
354 resources captured by our mass spectrometry and group possible substitutable resource; however,
we were unable to make positive identifications for many resource components in part due to the
356 low representation of environmental samples in the available databases. In addition, we tested for
patterns based on the polarity and molecular weight estimates but found no significant
358 relationships. This does not mean relationships between DOM and bacterial composition are

indescribable, but methods need to be developed to classify and group DOM components into
360 meaningful categories based on functional and metabolic forms.

362 **Generalist Communities**

Our results suggest that resource generalists may dominate many aquatic microbial communities,
364 and thus may explain why the effect of resource heterogeneity on community composition is
stronger in some lakes than others. Specifically, resource heterogeneity had a weak effect on the
366 composition of bacteria in lakes that separate along OTU PCoA Axis 2 (Fig. 3). Across our
lakes, we found a negative relationship between the abundance of generalists and the
368 concentrations of resources. This relationship is also correlated with the second axis of the DOM
PCoA, but not the first DOM PCoA axis which explains the majority of the DOM variation. One
370 possibility is that the majority of DOM resources are substitutable. Alternatively, consumers
could have multiple metabolic pathways for resource acquisition. For example, evidence from
372 comparative genomics suggests that aquatic bacteria capable of using complex organic matter
also have the potential to use numerous different resources, and may thus be generalists
374 (Livermore *et al.*, 2013; Newton *et al.*, 2010; Lauro *et al.*, 2009). As such, we propose that
resource generalists may be more common in aquatic ecosystems than previously thought
376 (Mariadassou *et al.*, 2015).

It is often assumed that most bacteria are specialists. For example, multiple studies have
378 identified taxa that specialize on particular resources (Hunt *et al.*, 2008; Mccarren *et al.*, 2010;
Gómez-Consarnau *et al.*, 2012; Jaspers and Overmann, 2004; Bird, 2012). The ability to use
380 multiple resources requires the production of extra enzymes and transporters; therefore, it is
costly to use numerous resources (Johnson *et al.*, 2012). As such, specialists may be

382 energetically favored in some environments. Likewise, numerous studies have indicated that
habitat specialists (e.g., sediment and aquatic) dominate bacterial communities (Székely and
384 Langenheder, 2013; Mariadassou *et al.*, 2015; Langenheder and Ragnarsson, 2007). However,
our results suggest that generalists are common in the lakes surveyed (Fig. 4). These findings are
386 supported by another study which found that resource generalists dominated coastal bacterial
communities (Mou *et al.*, 2008). It should be noted though, that we found both resource
388 generalists and specialists (Fig. S4) and therefore we are not suggesting that resource specialists
do not contribute to resource-diversity relationships. Instead, we argue that generalist may limit
390 the ability of resource heterogeneity to promote diversity when generalists are more dominant
than specialists.

392 We do acknowledge, however, that the method used to characterize DOM has some
limitations. First, the DOM extraction and detection may be biased towards some groups of
394 molecules (Dittmar *et al.*, 2008). While we may have missed some important components of the
DOM pool, we likely captured the complex terrestrial-derived organic matter that often
396 dominates aquatic ecosystems (Wilkinson *et al.*, 2013). This DOM has been shown to be
important for bacterial community structure and function (Lapierre *et al.*, 2013; Lennon and
398 Pfaff, 2005; Muscarella *et al.*, 2016). However, these are not the only important components of
the DOM pool, and we may have missed less complex labile molecules that can also affect
400 bacterial communities (Sarmiento and Gasol, 2012). However, many labile molecules would be
consumed rapidly, and thus we may not have been able to detect them. Second, our consumer-
402 resource interaction results are based on a single time point and therefore only suggest possible
bacteria-resource interactions. We use these correlations to make inferences about the degree to
404 which taxa are generalists. To make stronger inferences, we would need to conduct time-course

experiments during resource fluctuations and perform experimental manipulations of DOM
406 concentration and composition. Last, we assume that microbial communities are under local
selection due to resource availability, but other factors such as dispersal, predation, and the
408 physical environment can affect community composition. For example, high dispersal rates can
overwhelm local selection due to mass effects (Leibold *et al.*, 2004) which is especially
410 important in aquatic microbial communities that receive organisms from the neighboring
terrestrial landscape (Ruiz-Gonzalez *et al.*, 2015; Crump *et al.*, 2012). Regardless, our results,
412 and other genomic studies, suggest that resource generalist may dominate aquatic microbial
communities, and this should be investigated further.

414

Conclusions

416 Resource heterogeneity influenced the resource-diversity relationship and the
contribution of heterogeneity can be greater than concentration; however, when resource
418 generalists dominated communities the resource-diversity relationship was dampened. These
findings do not mean that there are no specialists in bacterial communities, because we find
420 evidence of resource specialist and others have found strong evidence for resource and habitat
specialists (Székely and Langenheder, 2013; Mariadassou *et al.*, 2015; Langenheder and
422 Ragnarsson, 2007; Bird, 2012; Muscarella *et al.*, 2016). These findings support the hypothesis
that generalist taxa may limit the affect resource heterogeneity has on local communities;
424 furthermore, we propose that consumer properties (i.e., generalist) and resource properties (i.e.,
availability) determine how strong communities respond to resource heterogeneity. In addition,
426 in order to understand how bacterial communities will respond to environmental changes, such
as changes in organic matter inputs due to changes in plant community distributions or global

428 climate change, we need to consider which resources are substitutable and which resources will
change in similar and predictive ways. In doing so, we will be able to understand how microbial
430 communities will respond to alterations in the available resources.

432

Acknowledgements: We acknowledge constructive feedback from AL Peralta and NI Wisnoski.

434 We thank BK Lehmkuhl for technical support and W Thorpe for logistical support. This work
was supported by the Huron Mountain Wildlife Foundation (MEM, JTL), the National Science
436 Foundation DEB-1442246 (JTL) & DEB-1501164 (MEM, JTL), and US Army Research Office
Grant W911NF-14-1-0411 (JTL). All code and data used in this study can be found in a public
438 GitHub repository (<https://www.github.com/LennonLab/ResourceHeterogeneity>) and the NCBI
SRA.

440

442 **Conflict of Interest:** The authors declare no conflict of interest

444

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- 644
- 646

TABLES

648 **Table 1.** Lake Properties and Chemistry – Latitude, longitude and surface area (hectares), pH,
650 temperature (Temp.), dissolved oxygen concentration (DO), chlorophyll *a* concentration (Chl *a*),
652 TN: total nitrogen, TP: total phosphorus, DOC: dissolved organic carbon.

Lake	Latitude	Longitude	Area (ha)	pH	Temp (°C)	DO (mg L ⁻¹)	Chl <i>a</i> (µg L ⁻¹)
Ann	46.8715	87.9220	25	7.86	27.44	7.22	1.25
Canyon	46.8334	87.9224	1.1	7.02	23.9	7.24	1.63
Howe	46.8916	87.9470	69	7.78	26.4	7.22	1.85
Ives	46.8440	87.8483	191	8.10	25.4	7.62	1.39
Lily	46.8475	87.8302	1.6	5.51	26.2	5.7	3.55
Mountain	46.8692	87.9063	338	8.31	26.5	7.93	2.14
Pony	46.8874	87.9175	0.5	5.39	25.3	7.04	16.35
Rush	46.8882	87.9067	127	8.14	25.7	7.74	1.23
Second Pine	46.8682	87.8572	69	8.09	26.2	7.17	3.76
Upper Pine	46.8624	87.8502	16	7.79	26.6	7.12	8.55

654

656 **Table 1 Cont.** Lake Chemistry. TN: total nitrogen, TP: total phosphorus, DOC: dissolved
658 organic carbon.

Lake	DOC (mg C L ⁻¹)	TP (μg P L ⁻¹)	TN (mg N L ⁻¹)
Ann	5.97	7.27	0.43
Canyon	7.23	2.64	0.38
Howe	7.04	5.21	0.57
Ives	6.91	9.15	0.38
Lily	14.35	11.55	0.93
Mountain	5.27	5.87	0.34
Pony	28.99	17.04	1.86
Rush	4.22	3.84	0.41
Second Pine	6.26	12.92	0.44
Upper Pine	7.84	11.21	0.57

660

FIGURE LEGENDS

662 **Fig. 1:** Principal coordinates analysis (PCoA) ordination of dissolved organic matter (DOM).
664 The distances between symbols represent the dissimilarity between DOM in each lake. Using
666 three axes, we can explain 71% of the variation in DOM composition. The third axis (not shown)
668 captures 14% of the variation. Symbol sizes reflect variation in the concentration of dissolved
organic carbon (DOC). Vectors represent the correlations between DOM composition and
various physical and chemical attributes of each lake including: pH, area, DOC, total nitrogen
(TN), total phosphorus (TP), and chlorophyll a (Chl).

670 **Fig. 2:** Bacterial community diversity relationships with resource concentration and resource
672 richness. Resource (DOC) concentration and species richness have been Box-Cox transformed to
meet model assumptions. There are significant positive relationships between species richness
674 and resource concentration and between species evenness and dissolved organic matter (DOM)
richness. There are significant negative relationships between species evenness and resource
676 concentration and between species richness and DOM richness. Dashed line represents linear
regression fit along with 95% confidence intervals.

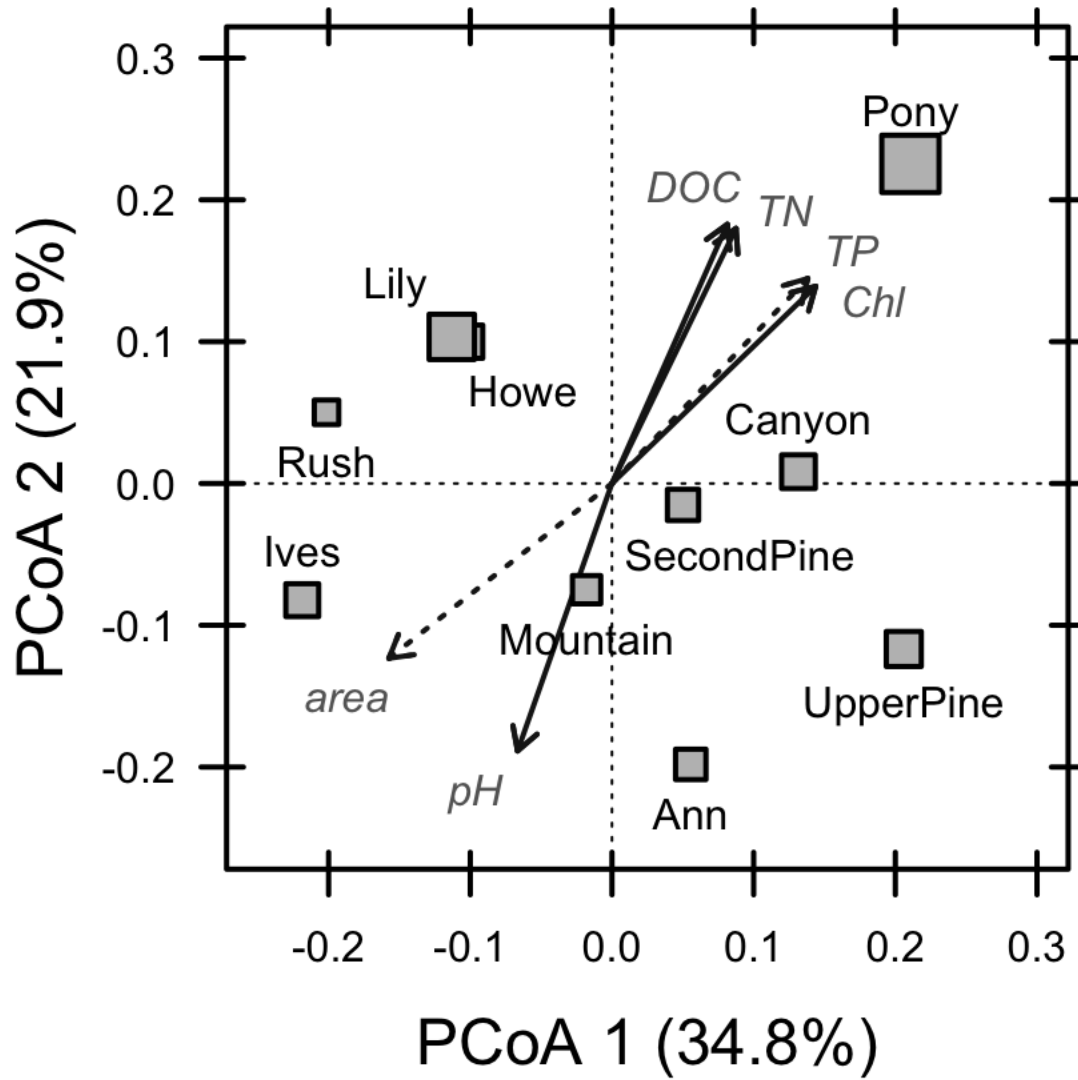
678 **Fig. 3:** Principal coordinates analysis (PCoA) ordination of bacterial communities. Vectors
680 represent the correlation between the dissolved organic matter (DOM) heterogeneity and the
bacterial community composition. The two vectors are based on correlations between community
682 composition and the site scores from the DOM PCoA axes one and two. We used distance-based
redundancy analysis to test the relationship between DOM site scores and bacterial community
684 composition.

686 **Fig. 4:** The proportion of generalists and cosmopolitan taxa in aquatic bacterial communities. We
defined operational taxonomic units (OTUs) as generalists using consumer-resource co-
688 occurrence (top) and as cosmopolitan based on spatial occurrence (top). We used OTU relative
abundances to calculate the proportion in each community. For both, we used a linear model to
690 determine if there was a relationship between the proportion of generalists and the concentration
of dissolved organic carbon (DOC). For both, we found a significant negative relationship.
692 Dashed line represents linear regression fit along with 95% confidence intervals. The light gray
dotted line represents 50% of the community and is used as a reference.
694

FIGURES

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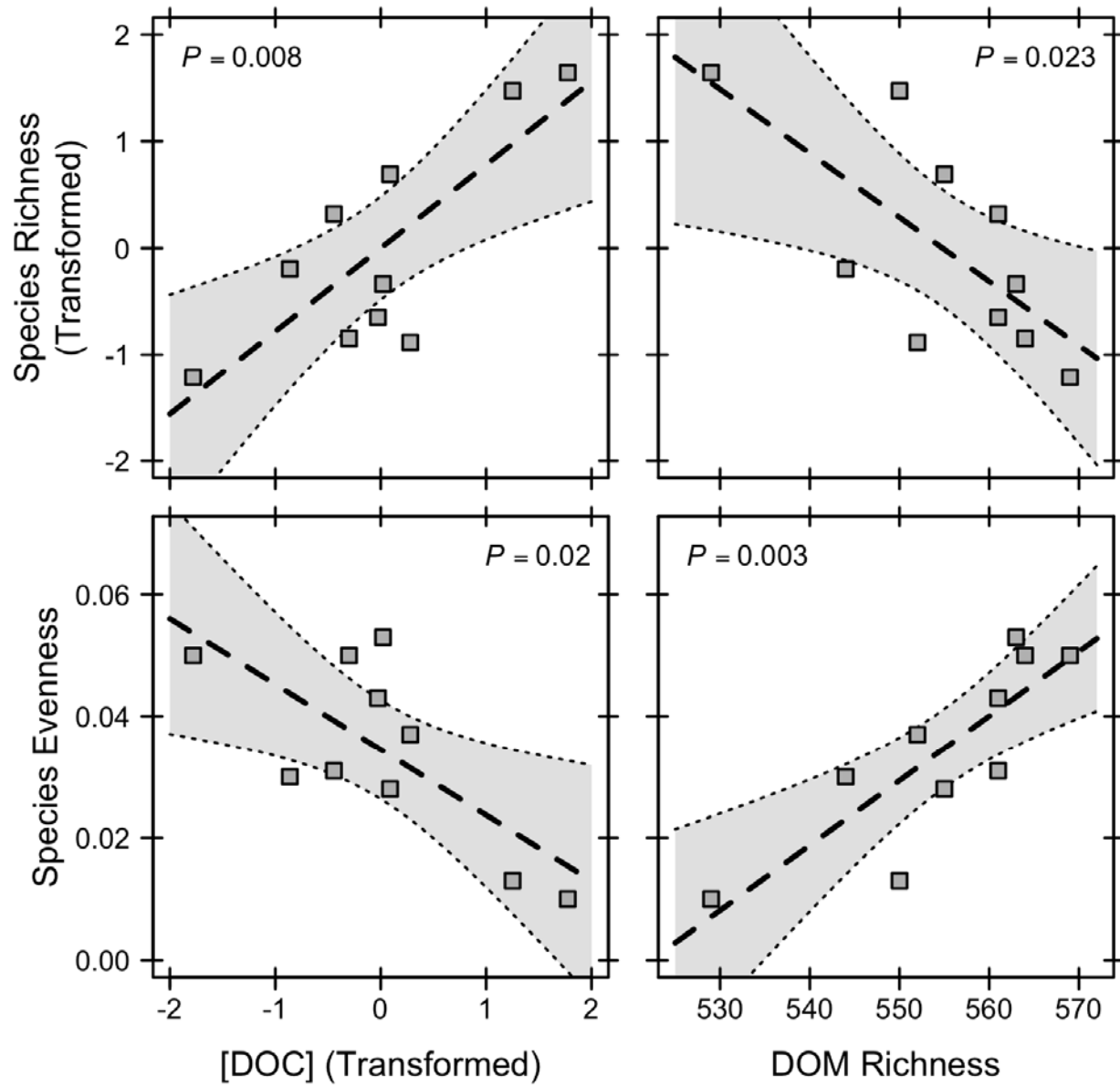
698 **Figure 1:**



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702 **Figure 2:**

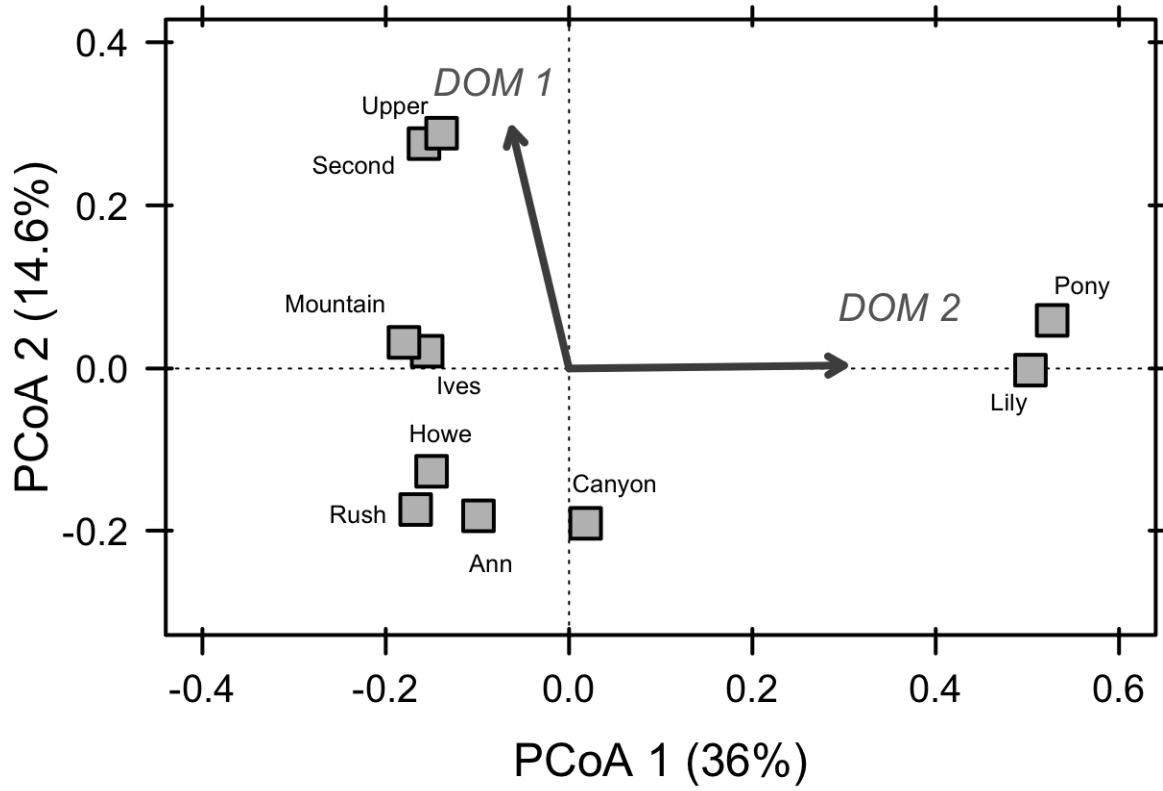
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Figure 3:

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Figure 4:

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