

2                    **Species sorting along a subsidy gradient alters community stability**

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14

## ABSTRACT

16 The movement of resources between terrestrial and aquatic habitats has strong effects on  
ecological processes in recipient ecosystems. Allochthonous inputs modify the quality and  
18 quantity of the available resource pool in ways that may alter the composition and stability of  
recipient communities. Inputs of terrestrial dissolved organic carbon (tDOC) into aquatic  
20 ecosystems represent a large influx of resources that has the potential to affect local  
communities, especially microorganisms. To evaluate the effects terrestrial inputs on aquatic  
22 microbial community composition and stability, we manipulated the supply rate of tDOC to a set  
of experimental ponds. Along the tDOC supply gradient, we measured changes in diversity and  
24 taxon-specific changes in abundance and activity. We then determined community stability by  
perturbing each pond using a pulse of inorganic nutrients and measuring changes in composition  
26 and activity (i.e., responsiveness) along the gradient. Terrestrial DOC supply significantly altered  
the composition of the active microbial community. The composition of the active bacterial  
28 community changed via decreases in richness and evenness as well as taxon-specific changes in  
abundance and activity indicating species sorting along the gradient. Likewise, the  
30 responsiveness of the active bacterial community decreased along the gradient, which led to a  
more stable active community. We did not, however, observe these changes in diversity and  
32 stability in the total community (i.e., active and inactive organisms), which suggests that tDOC  
supply modifies microbial community stability through functional not structural changes.  
34 Together, these results show that altered aquatic terrestrial linkages can have profound effects on  
the activity and stability of the base of the food web and thus can alter ecosystem functioning.

36

## INTRODUCTION

38 Resource subsidies refer to the cross-boundary movement of materials and energy from  
donor to recipient ecosystems. These allochthonous inputs affect various ecological processes  
40 including population dynamics, species interactions, and ecosystem functioning (Polis et al.  
1997, Holt 2004). Subsidies can also alter the stability of recipient communities. Theory suggests  
42 that low to moderate levels of subsidies stabilize recipient communities by promoting trophic  
omnivory (Polis et al. 1997, Huxel and McCann 1998) and dampening oscillations between  
44 consumers and their resources (DeAngelis 1992, Huxel and McCann 1998). The precise effect of  
subsidies on recipient communities, however, likely depends on the timing (Takimoto 2002),  
46 quality (Bartels et al. 2012), and quantity (Cottingham and Narayan 2013). Likewise, subsidies  
can also affect stability by altering the composition of recipient communities by selecting for and  
48 against particular species (Holt 2004). If subsidies alter communities via species sorting, then  
they will also alter the distribution of species-specific traits that may affect stability, including  
50 resource specialization (Huxel and McCann 1998, Faria and Costa 2010) and metabolic plasticity  
(Comte et al. 2013). Together, the effect of subsidies on community stability is likely influenced  
52 by both subsidy properties (e.g., quantity) and consumer traits (e.g., resource specialization).

A well-recognized and pervasive subsidy is the movement of dissolved organic carbon  
54 (DOC) from terrestrial to aquatic ecosystems (Polis et al. 1997, Tranvik et al. 2009). In most  
inland water bodies, terrestrial DOC (tDOC) inputs exceed internal (i.e., autochthonous) inputs  
56 by aquatic autotrophs by at least an order of magnitude (Tranvik et al. 2009, Wilkinson et al.  
2013). Additionally, there is growing evidence that tDOC inputs are increasing in many regions  
58 around the world owing to global change (Monteith et al. 2007), and it has been hypothesized  
that such changes in tDOC inputs will affect the functioning and stability of aquatic ecosystems

60 (Jones and Lennon 2015, Solomon et al. 2015). Heterotrophic microorganisms are the primary  
consumers of tDOC in aquatic ecosystems. Despite having recalcitrant properties, tDOC is used  
62 by bacteria for anabolic and catabolic processes (Kritzberg et al. 2004, Lapierre et al. 2013). As  
such, heterotrophic bacteria are expected to mediate the aquatic ecosystem response to tDOC  
64 subsidies via changes in community composition and metabolic activity (Jones et al. 2009,  
Comte and del Giorgio 2011), and it has been hypothesized that, due to these changes, subsidies  
66 may alter the stability of recipient aquatic ecosystems (Wetzel 1999).

If subsidies modify the composition of recipient communities through processes such as  
68 species sorting, then subsidies could also alter the function and stability of recipient  
communities. Microbial communities are comprised of species with traits that link ecosystem  
70 functioning and community stability. For example, the degree to which communities are  
dominated by either generalist or specialists has important consequences for carbon cycling  
72 (Mou et al. 2008) and may explain how microbial communities respond to perturbations (Allison  
and Martiny 2008, Shade et al. 2011). Another important trait is metabolic plasticity or the  
74 ability of taxa to change physiological functions or to transition across levels of metabolic  
activity (e.g., active to dormant). Microbial communities consist of taxa that differ in activity and  
76 their degree of metabolic plasticity (del Giorgio and Gasol 2008, Lennon and Jones 2011), and  
the ability of individual taxa to rapidly adjust metabolic activity has been shown to be important  
78 for controlling ecosystem functions such as carbon and nitrogen cycling (Aanderud et al. 2015).  
In addition, the ability of taxa to adjust metabolic functions (Comte et al. 2013) or to transition  
80 across levels of activity (Lennon and Jones 2011) have been hypothesized to buffer communities  
against perturbations. Therefore, processes such as species sorting will alter the distribution of  
82 taxa and traits within communities and thus affect the stability and functioning of ecosystems.

In this study, we quantified the effects of tDOC supply on the diversity, composition, and  
84 stability of aquatic microbial communities. We hypothesized that subsidies would change  
bacterial composition via shifts in either the abundance or activity of taxa which would be  
86 reflective of species sorting. We further hypothesized that resource-driven shifts in composition  
would determine how subsidized bacterial communities respond to perturbations. To test our  
88 hypotheses, we manipulated tDOC supply rate in eleven experimental ponds. First, we used  
microbial community sequencing to assess changes in diversity and trends in abundance and  
90 activity across experimental treatments. Then, we measured community stability by quantifying  
changes in community composition in each pond following an inorganic nutrient perturbation.  
92 Results from our study provide a framework for how aquatic microbial communities may  
respond to increased resource subsidies such as tDOC, and show that subsidy-mediated shifts in  
94 composition alter the stability of communities.

## 96 MATERIALS AND METHODS

*Experimental Ponds* – We manipulated the supply rate of terrestrial dissolved organic carbon  
98 (tDOC) in eleven experimental ponds at the Michigan State University’s W.K. Kellogg  
Biological Station (KBS) Experimental Pond Facility. Each experimental pond has a 30 m  
100 diameter, a 2 m maximum depth, and an operating volume of approximately  $10^6$  L. We  
established a tDOC supply gradient by adding different amounts of a commercially available  
102 source of humic substances (Super Hume, Crop Master USA) to each pond using a 5 horsepower  
trash pump. This source of humic substances is comprised of 17% humic acid and 13% fulvic  
104 acid and is known to be used by diverse heterotrophic bacteria (Lennon et al. 2013). We  
maintained the tDOC supply gradient for 100 days (6 June 2009 to 14 September 2009) by

106 adding Super Hume to each pond on a weekly basis assuming a loss rate of  $4.7 - 12.2\% \text{ d}^{-1}$   
(Lennon et al., 2013; Jones & Lennon, 2015).

108

***Perturbation and Sampling*** – Nutrient limitation is typical for inland water bodies including the  
110 KBS experimental ponds, and aquatic communities are sensitive to episodic nutrient inputs  
(Elser et al. 1990). As such, nutrient pulses are a common perturbation to aquatic ecosystems and  
112 are often used in experiments to test questions about stability (Nowlin et al. 2008). We used an  
inorganic nutrient pulse to test the stability of aquatic microbial communities along the tDOC  
114 supply gradient. We added 500 L of an inorganic nutrient solution ( $\text{NH}_4\text{NO}_3$  and  $\text{Na}_2\text{HPO}_4$ ) to  
each experimental pond on day 82 (27 Aug 2009) using a 5 horsepower trash pump. The  
116 inorganic nutrient pulse elevated inorganic nitrogen (N) and phosphorus (P) concentrations of  
each pond by  $565 \mu\text{g L}^{-1}$  and  $50 \mu\text{g L}^{-1}$ , respectively, while maintaining the initial N:P molar  
118 ratio. Prior to and after the inorganic nutrient pulse, we sampled each pond three times per week  
to track changes in water chemistry. We collected water samples from the center of each pond  
120 using a 1 m depth-integrated sampler. We measured DOC by oxidation and nondispersive  
infrared (NDIR) detection using a Shimadzu TOC-V carbon analyzer on  $0.7 \mu\text{m}$  (Whatman,  
122 GF/F) filtered water samples. We measured total nitrogen concentrations spectrophotometrically  
after persulfate digestion using the second-derivative method (APHA 1998). We measured  
124 soluble reactive phosphorus concentrations colorometrically using the ammonium molybdate  
method (Wetzel and Likens 2000). Further details about chemical analyses and the nutrient  
126 perturbation can be found elsewhere (Jones and Lennon 2015).

128 ***Bacterial Community Characterization*** – We characterized aquatic bacterial composition along  
the tDOC supply gradient using 16S rRNA sequencing approximately a week prior to (day 74)  
130 and after (day 88) the inorganic nutrient pulse. We collected water samples from the center of  
each pond using a 1 m depth-integrated sampler. We retained microbial biomass on 47 mm 0.2  
132  $\mu\text{m}$  Supor PES membrane filters (Pall) and stored immediately at  $-80\text{ }^{\circ}\text{C}$ . Because  
microorganisms exist at various levels of metabolic activity which have differential effects on  
134 the structure and function of the community (del Giorgio and Gasol 2008), we used two  
approaches to characterizing composition. One approach used DNA, a stable molecule, to  
136 characterize the microbial community based on all taxa, regardless of activity level. We refer to  
the DNA approach as the “total community”. The second approach used RNA, an ephemeral  
138 molecule reflecting metabolic growth and activity (Elser et al. 2003), to characterize the  
microbial community based on the organisms that contribute to ecosystem function (Jones and  
140 Lennon 2010, Aanderud et al. 2015). We refer to the RNA approach as the “active community”.  
We extracted nucleic acids (DNA and RNA) using the PowerSoil Total RNA Extraction Kit with  
142 DNA Elution Accessory Kit (MoBio, Carlsbad, CA). We treated RNA extracts with DNase  
(Invitrogen) to degrade DNA prior to cDNA synthesis via the SuperScript III First Strand  
144 Synthesis Kit (Invitrogen). Once DNA and cDNA samples were cleaned and quantified, we  
amplified the 16S rRNA gene (DNA) and transcript (cDNA) using barcoded primers (515F and  
146 806R) designed to work with the Roche 454 sequencing platform (Fierer et al. 2008; see  
Supplement for PCR sequences and conditions). We sequenced 16S rRNA amplicons at the  
148 Research Technology Support Facility at Michigan State University using the GS FLX Titanium  
chemistry. We processed raw 16S rRNA sequences using the software package *mothur* (version  
150 1.32.1, Schloss et al. 2009). To identify operational taxonomic units (OTUs), we binned

152 sequences into phylotypes using the Ribosomal Database Project's 16S rRNA database and taxonomy as the reference (Cole et al. 2009).

154 **Community Diversity** – To determine the effects of tDOC supply on bacterial community diversity, we measured alpha diversity in each pond prior to the inorganic nutrient pulse. First, we estimated taxonomic richness using a resampling approach. We subsampled communities to obtain a standardized 2000 observations per site and summed the number of OTUs represented in each subsample. We then resampled 999 additional times and calculated an average richness estimate ( $\pm$  SEM) for each site (Muscarella et al. 2014). We used Good's Coverage to confirm that subsampling to 2000 observations was sufficient to make conclusions based on our data set (Good, 1953). Second, we determined taxonomic evenness using Simpson's Evenness (Smith and Wilson 1996). Evenness was calculated using the same resampling approach we used for richness. We calculated richness and evenness for the total (DNA) and active (RNA) microbial communities. For each metric, we used an indicator variable multiple regression model (Neter et al. 1996) to test how diversity changed in response to tDOC supply with respect to both the total (DNA) and active (RNA) microbial community. In our regression model, we used supply rate as the continuous variable and community type (total vs. active) as the categorical variable. All statistical calculations were performed in the R computing environment (v 3.1.3, R Core Development Team 2012).

170

172 **Community Composition and Species Sorting** – To determine the effects of tDOC supply on community composition and test for evidence of species sorting, we determined how bacterial communities and individual bacterial taxa responded to the tDOC gradient. First, we used



174 PERMANOVA to determine if the bacterial community responded to tDOC supply rate for both  
the total and active communities (Anderson 2001). For each, if the PERMANOVA was  
176 significant we tested for evidence of species sorting defined here as species-specific directional  
changes in abundance (Jablonski 2008). We used Spearman's rank-order correlation to measure  
178 the response of each taxon to tDOC supply. We identified responsive taxa based on significant  
rho-values after a Benjamini-Hochberg correction for multiple comparisons (Benjamini and  
180 Hochberg 1995). Positive rho-values indicate taxa that responded positively (in either the total or  
active community) to tDOC supply and negative rho-values identified taxa that responded  
182 negatively to tDOC supply. We then used the Ribosomal Database Project's Taxonomy (Cole et  
al. 2009) and a curated freshwater bacteria database (Newton et al. 2011) to taxonomically  
184 identify each responsive taxa. All statistical calculations were performed in the R computing  
environment and PERMANOVA was implemented using the *adonis* function in the R package  
186 *vegan* (v 2.2-1; Oksanen et al. 2013). Taxonomic identifications were performed using *mothur*.

188 **Community Stability** – We determined the effects of the subsidy supply on community stability  
by calculating the change in community composition before and after the inorganic nutrient  
190 pulse (He et al. 1994, Brown 2003). First, we used Principal Coordinates Analysis (PCoA) to  
visualize differences in community composition based on Bray-Curtis distances. PCoA is a  
192 metric multidimensional scaling technique that allows objects to be oriented in a common  
reduced-dimension space while maintaining distance between objects as best as possible  
194 (Legendre and Legendre 2012). We used  $\log_{10}$ -transformed relative abundances and Bray-Curtis  
distance to reduce bias against low abundance taxa (Anderson et al. 2006, Legendre and  
196 Legendre 2012). We then measured the Euclidean distance in multivariate space between paired

communities before and after the inorganic nutrient pulse using the first three multivariate axes  
198 (Brown, 2003). The Euclidean distance estimates the overall change in the microbial community,  
and we used the distance, which we refer to as “responsiveness”, as a metric of community  
200 stability (Grimm and Wissel 1997). Thus, a more stable community would be one that is less  
responsive to the inorganic nutrient pulse. We used an indicator variable multiple regression to  
202 test how subsidy supply rate altered community stability (i.e., responsiveness) with respect to  
both the total (DNA) and active (RNA) microbial communities. In our regression model, we used  
204 supply rate as the continuous variable and community type (total vs. active) as the categorical  
variable. All statistical calculations were performed in the R computing environment and PCoA  
206 was implemented using the *cmdscale* function in the R package *vegan*.

## 208 **RESULTS**

***DOC Manipulation and Nutrient Perturbation*** – The tDOC supply rates imposed (0 – 200 g  
210 C/m<sup>2</sup>) reflect the range of tDOC received by temperate lakes under current and future expected  
supply rates (Solomon et al. 2015), and established a DOC concentration gradient across ponds  
212 from 6 to 24 mg C/L (Supplemental Fig. 1). After the tDOC supply gradient had been established  
for 80 days, the inorganic nutrient pulse rapidly elevated nutrient concentrations approximately  
214 10-fold while maintaining N:P ratios observed prior to the perturbation (Supplemental Fig. 2).

***Community Diversity*** – The tDOC supply gradient significantly decreased the richness and  
evenness of the active microbial community but had no effect on the diversity of the total  
218 community. An indicator variable multiple regression revealed that tDOC supply rate explained  
85% of the observed variation in bacterial richness ( $F_{3,15} = 34.2$ ,  $P < 0.001$ ). Based on the total

220 community (i.e., all taxa), richness did not change in response to tDOC supply ( $P = 0.50$ );  
however, the richness of the active community decreased in response to tDOC supply. Overall,  
222 we observed a 30% drop in the number of active taxa across the gradient (Fig. 1A;  $R^2 = 0.64$ ,  $P =$   
0.001). Similarly, an indicator variables multiple regression model revealed that tDOC supply  
224 rate explained 54% of the observed variation in community evenness ( $F_{3, 15} = 8.12$ ,  $P = 0.002$ ).  
Based on the total community, community evenness did not change in response to tDOC supply  
226 ( $P = 0.32$ ); however, the evenness of the active community decreased in response to tDOC  
supply with a 25% drop in evenness across the entire gradient (Fig. 1B;  $R^2 = 0.51$ ,  $P = 0.04$ ).

228  
***Community Composition and Species Sorting*** – The tDOC supply gradient had a significant  
230 effect on community composition for both the total and active communities. Our PERMANOVA  
results show that tDOC supply altered the composition of the total ( $R^2 = 0.17$ ;  $P = 0.03$ ) and  
232 active ( $R^2 = 0.15$ ,  $P = 0.04$ ) bacterial community. In addition, we found evidence of species-  
specific responses in both the total and active communities. Together, 29 bacterial taxa (24% of  
234 the observed OTUs representing 26% of the total sequences) demonstrated a significant  
directional response based on the total (i.e., DNA) or active (i.e., RNA) community. Based on  
236 DNA analysis, 19 taxa significantly correlated with tDOC supply with eight positive and 11  
negative relationships (Fig. 2B). Based on RNA analysis, 15 taxa significantly correlated with  
238 tDOC supply with one positive relationship and 14 negative relationships (Fig. 2C). The other  
121 bacterial taxa did not demonstrate a significant relationship because their relative  
240 abundances were either constant or changed sporadically across the tDOC supply gradient.

242 **Community Stability** – The tDOC supply gradient significantly enhanced the stability of the  
active microbial community but had no effect on the stability of the total community. For our  
244 PCoA analysis, the first three ordination axes explained 60% of the variation between microbial  
communities across the tDOC supply gradient. An indicator variable multiple regression  
246 revealed that tDOC supply rate and community type (total or active) explained 83% of the  
observed variation in responsiveness ( $F_{3, 18} = 35$ ,  $P < 0.001$ ). The responsiveness of the total  
248 community was low and did not change across the tDOC supply gradient ( $P = 0.37$ , Fig. 3A).  
However, the active community became less responsive as tDOC supply increased ( $P < 0.001$ ,  
250 Fig. 3A), and there was a 35% drop in responsiveness across the entire gradient.

## 252 DISCUSSION

The cross boundary movement of resources can alter the composition and stability of  
254 recipient communities, and therefore change community dynamics and ecosystem functioning. In  
this study, we manipulated the supply rate of terrestrial dissolved organic carbon (tDOC) to  
256 aquatic ecosystems and documented changes in the composition and stability of aquatic  
microbial communities. We found that the subsidy selected for and against certain taxa and  
258 suggest that this is evidence of tDOC-mediated species sorting. In addition, we found that supply  
rate increased the stability of the active members of the microbial community, and propose that  
260 via species sorting, tDOC established an active community that was less responsive to an  
inorganic nutrient pulse. Together, our results suggest that in the face nutrient perturbations,  
262 some subsidies (e.g. tDOC) select for specialized taxa that increase community stability.  
Furthermore, because these subsidies stabilize the organisms that regulate ecosystem  
264 functioning, the ability of ecosystems to contend with nutrient perturbations may diminish due to

a reduced capacity for structural and functional responses by the members of the microbial  
266 community.

### 268 **Effects of terrestrial DOC on aquatic bacterial diversity**

The tDOC supply gradient altered the aquatic microbial community by decreasing the  
270 richness and evenness of the active taxa. This suggests that ecosystems receiving high inputs of  
tDOC contain fewer active taxa and that certain taxa are disproportionately responsible for the  
272 majority of microbial activity in these ecosystems. There are multiple mechanisms by which  
increased tDOC inputs could decrease the diversity of active bacteria. First, tDOC is  
274 chromophoric and can decrease light availability, limit aquatic primary productivity, and thus  
reduces the concentration of labile, algal-derived resources that are used by many aquatic  
276 bacteria (Karlsson et al. 2009, Lennon et al. 2013, Jones and Lennon 2015). The decreased inputs  
of autochthonous inputs would have negative effects on aquatic microorganisms that rely on  
278 algal-derived resources (Kritzberg et al. 2004), and thus decrease the richness of the active  
community. Second, tDOC is a chemically and physically recalcitrant resource pool (Fellman et  
280 al. 2009), and it has been argued that only specialized consumers use tDOC (Fuchs et al. 2011).  
For example, the degradation of aromatic compounds requires specific metabolic pathways, most  
282 notably the beta-ketoadipate pathway (Fuchs et al. 2011). Therefore, tDOC subsidies may only  
favor taxa with specialized metabolic pathways (Mou et al. 2008, Fuchs et al. 2011). If these  
284 pathways are rare in the community then only few taxa would benefit from the chemical niches  
offered by tDOC. In sum, the disproportional benefit of tDOC would decrease the richness and  
286 evenness of the active microbial community.

288 **Species sorting alters community structure**

290 In addition to changes in diversity, the altered resource environment modified the  
292 composition of aquatic bacterial communities by selecting for and against certain taxa (i.e.  
294 species sorting). We observed significant increases and decreases in taxa along the resource  
296 gradient. Although comparative studies have observed that DOC is an important factor  
structuring bacterial communities (Judd et al. 2006, Jones et al. 2009), here we present some of  
the first experimental evidence that tDOC supply can structure aquatic bacterial communities by  
species sorting.

296 In this study, we observed that some taxa increase in abundance in response to tDOC supply  
while other taxa decrease in abundance. For example, there was a 10-fold increase in the relative  
298 abundance of OTU008 (*Methylomonas* sp.) and a 10-fold decrease in the relative abundance of  
OTU011 (*Rhodococcus* sp.) across the gradient. There are multiple potential explanations for the  
300 taxon-specific changes in relative abundance we observed. First, the subsidy could be selecting  
against taxa unable to use tDOC while other taxa remain constant. For example, in addition to  
302 *Rhodococcus* (OTU011), four taxa belong to the Verrucomicrobia clade decreased in abundance  
and activity across the gradient (see Supplemental Table 1). In general, the Verrucomicrobia  
304 clade is thought to primarily use labile, algal-derived carbon (Newton et al. 2011). Second, taxa  
with beneficial traits (e.g., specialized metabolic functions) could be increasing in abundance  
306 while other taxa remain constant. For example, one of the strongest responding taxa in our study  
was an Actinobacterium (OTU043) related to the freshwater Actinobacteria tribe acSTL; in  
308 addition, we found increases in abundance for other Actinobacteria including members of the  
Luna3 and acIII tribes (see Supplemental Table 1). Though few cultured representatives exist,  
310 many members of the phylum Actinobacteria are known for the degradation of complex organic

312 matter (Newton et al. 2011, Nelson and Carlson 2012). Last, because tDOC supply can promote  
314 additional microbial metabolisms, distinct groups of microorganisms may benefit from increased  
316 tDOC supply. For example, high concentrations of DOC may enhance lake methane cycling  
(Bastviken 2004, Lennon et al. 2006). We observed an increase in the relative abundance  
*Methylomonas* (OTU008) across the tDOC supply gradient, and this organism obtains carbon  
and energy from methane (i.e., methanotrophy). Together, our results provide evidence that  
subsidies, such as tDOC, structure bacterial communities via species sorting.

318

### **Microbial Generalists and Specialists**

320 In our study, we observed taxon-specific shifts in relative abundances, which indicate tDOC  
may favor taxa with specialized metabolic traits. Theory suggest that habitats with low  
322 concentrations of growth-limiting resources are thought to select for specialists that have lower  
minimal resource requirements, while habitats with higher concentrations of growth limiting  
324 resources may select for generalists (Wilson and Yoshimura 1994). In contrast, we increased the  
supply rate of tDOC, a low-quality complex resource requiring specialized metabolic traits, and  
326 observed taxon-specific increases in abundance. If these taxa are using tDOC as a resource, we  
can assume that they possess the required metabolic pathways and are specialists. Alternatively,  
328 these organisms may not be specialists. Work in marine environments has found that generalist  
microbes containing the diverse metabolic pathways are responsible for using tDOC (Mou et al.  
330 2008, Newton et al. 2010). Likewise, work in the field of comparative genomics has shown that  
organisms that use large carbon resources tend to have larger genomes, which is a common  
332 signature of generalist life-history strategies among microorganisms (Livermore et al. 2014). It is  
possible, therefore, that some taxa contain specialized metabolic pathways but are in fact carbon

334 substrate generalists rather than specialists. Therefore, we may need to change our expectations  
of the resource availability generalist-specialist framework to include resource complexity.  
336 When resources are available to all consumers (low complexity) it is clear that resource  
availability will favor the consumers with the lowest minimal resource requirements (Tilman  
338 1977); however, when resources are limited to consumers with unique traits (complex resources)  
then resource availability will favor consumers possessing these traits. Though often ignored,  
340 many nutrients – such as nitrogen, phosphorus, and carbon – exist as heterogeneous mixtures of  
molecules that differ in quality and bioavailability (Muscarella et al. 2014). As such, it is  
342 important to consider properties such as quality when predicting how resource subsidies will  
favor generalists versus specialists in a community.

344

### **Community Response to Perturbations**

346 In addition to compositional changes, tDOC supply rate altered how the bacterial community  
responded to an inorganic nutrient perturbation. There are four main predictions for how  
348 microbial communities will respond to perturbations: 1) resistant communities will show no  
changes; 2) resilient communities will quickly recover after perturbations and resume pre-  
350 disturbance structure and function; 3) sensitive, functionally redundant communities will change  
but maintain pre-disturbance function, and 4) sensitive, non-redundant communities will change  
352 in composition and function (Allison and Martiny 2008). Previous studies using nutrient  
perturbations have shown that microbial composition is generally sensitive to perturbation  
354 (Allison and Martiny 2008). This is not what we observed. In our study we found that the total  
bacterial communities had little to no response to a nutrient perturbation regardless of tDOC  
356 supply rate. This indicates that the total community was either resistant or resilient. However, we



found that the active community changed across the tDOC supply gradient and that the active  
358 taxa responded less to the nutrient perturbation as tDOC supply increased. Because RNA is more  
ephemeral than DNA, we can assume that, if the community changed, the total community  
360 (based on DNA) would not return to pre-perturbation composition before the active community  
(based on RNA). Therefore, taking both the total and active communities into account, we argue  
362 that overall the total bacterial community was resistant to the inorganic nutrient pulse but the  
metabolically active taxa responded. In addition, the stability (i.e., resistance) of the  
364 metabolically active taxa increased as tDOC supply rate increased. We propose that the observed  
changes in the active community represent the functional responsiveness and metabolic plasticity  
366 of the bacterial community. Because only the functionally active organisms responded, the  
community response to the inorganic nutrient pulse was functional not structural; furthermore,  
368 because the functional responsiveness decreased as tDOC supply rate increased, subsidy inputs  
yielded a microbial community that was structurally and functionally stable.

370

### **Subsidies and Community Stability**

372 Overall, experimental tests to determine the effects of resource subsidies on community stability  
have been sparse (Nowlin et al. 2007). In this study, we found that a tDOC subsidy stabilized the  
374 recipient community by altering the composition of the microbial community through species  
sorting. One mechanism would be that the subsidy favored specialized taxa. However, due to  
376 their metabolic physiology, these specialized taxa may be relatively slow growing and unable to  
rapidly respond to nutrient pulses (Wetzel 1999). An alternative mechanism would be that the  
378 subsidy selected against fast-responding taxa. For example, at high tDOC supply shading limits  
primary productivity and the aquatic microbial community may receive less labile algal-derived

380 carbon to promote fastidious, rapid responding taxa (Jones and Lennon 2015). Our data suggest  
that both of these mechanisms explain changes in composition due to tDOC supply. Therefore,  
382 we hypothesize that the quality of the subsidy influences community stability. Here, we added a  
low quality subsidy (tDOC) which selected for taxa with specialized metabolic functions and  
384 against fastidious taxa which would have been able to rapidly respond to the inorganic nutrient  
pulse. Conversely, systems receiving high-quality subsidies would favor more responsive taxa  
386 and thus communities would respond differently to perturbations. Therefore, subsidies affect  
community stability by altering the composition of the community through species sorting, but  
388 the community stability outcome will be dependent on the properties of the subsidy and the traits  
of the recipient consumers.

390

### **Implications for Aquatic Ecosystems**

392 Overall, our findings suggest that the active taxa within the microbial community are  
responsible for mediating the changes in composition and stability due to altered subsidy inputs.  
394 Because the active taxa control nutrient cycling, we also expect these changes to affect  
ecosystem function and stability. First, subsidy-induced changes in community composition will  
396 alter ecosystem functions. For example, the degree to which bacterial communities are  
physiologically flexible may have important consequences for ecosystem functions such as  
398 secondary productivity (Godwin and Cotner 2015), and our data suggest that tDOC subsidies  
may select for communities comprised of taxa that are less physiological flexibility and thus  
400 unable to rapidly respond to nutrient pulses. Second, changes in community stability may alter  
ecosystem stability. For example, enhanced community stability may yield reduced ecosystem  
402 stability because either functional or compositional changes are required for the ecosystems to

respond to the nutrient perturbations (Comte and del Giorgio 2011). If inorganic nutrients from a  
404 pulse perturbation remain in the recipient ecosystem, due to slow rates of biological processing,  
ecosystem functioning will become destabilized (Cottingham and Carpenter 1994). In fact,  
406 previous work demonstrated that tDOC inputs diminished nutrient turnover time and thus  
destabilized aquatic ecosystem functioning (Jones and Lennon 2015). We hypothesized that the  
408 reduced ecosystem stability was due to a lack of microbial functional or structural responses. Our  
results confirmed this hypothesis. Together, our results support the view that subsidy supply  
410 alters species sorting in ways that alter the stability of recipient communities and ecosystems.

412

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416 earlier version of this manuscript. All sequence data and metadata have been submitted to NCBI  
and are available at BioProject PRJNA301893. All code for sequence processing and statistical  
418 analyses is available at <https://github.com/LennonLab/SubsidyMicrobialStability>.

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## FIGURE LEGENDS

**Fig. 1:** Community diversity estimates, richness (A) and evenness (B), for microbial

586 communities in response to tDOC supply rate. For both, the diversity of the total community (DNA, circles) did not change in response to tDOC supply rate (A:  $P = 0.50$ ; B:  $P = 0.32$ ).

588 However, both the richness and evenness of the active community (RNA, triangles) decreased in response to tDOC supply rate (A:  $P = 0.001$ ; B:  $P = 0.04$ ).

590 **Fig. 2:** Taxon-specific changes in community composition along the tDOC supply rate gradient.

A) Phylogenetic tree of responsive taxa as indicated by significant Spearman's rank correlations.

592 The consensus tree was inferred by using the Maximum Likelihood methods based on the Jukes-Cantor model, and 100 bootstraps using MEGA-6 (Tamura et al. 2013). Responses indicate

594 significant increases ( $\uparrow$ ) or decreases ( $\downarrow$ ) in relative abundance in as tDOC supply rate increased.

Responses are shown in regards to both the active and total communities. Clade refers to the

596 phylogenetic group (phylum or subphylum) as inferred from best matches to the RDP taxonomy (Wang et al. 2007). Classifications based on known freshwater bacteria are found in

598 Supplemental Table I. B) Example of significant positive correlation as shown by OTU008

(*Methylomonas* sp.). C) Example of a significant negative correlation as shown by OTU011

600 (*Rhodococcus* sp.). For each, log relative abundances are shown for OTU relative abundance in

the total communities. Dashed lines indicate LOWESS regression trends and are included to

602 demonstrate overall trends.

**Fig. 3:** Community stability to an inorganic nutrient perturbation along the tDOC supply rate.

604 Responsiveness was measured as the Euclidian distance between paired pre- and post-

perturbation communities in multivariate space using the first three axes. Lower responsiveness

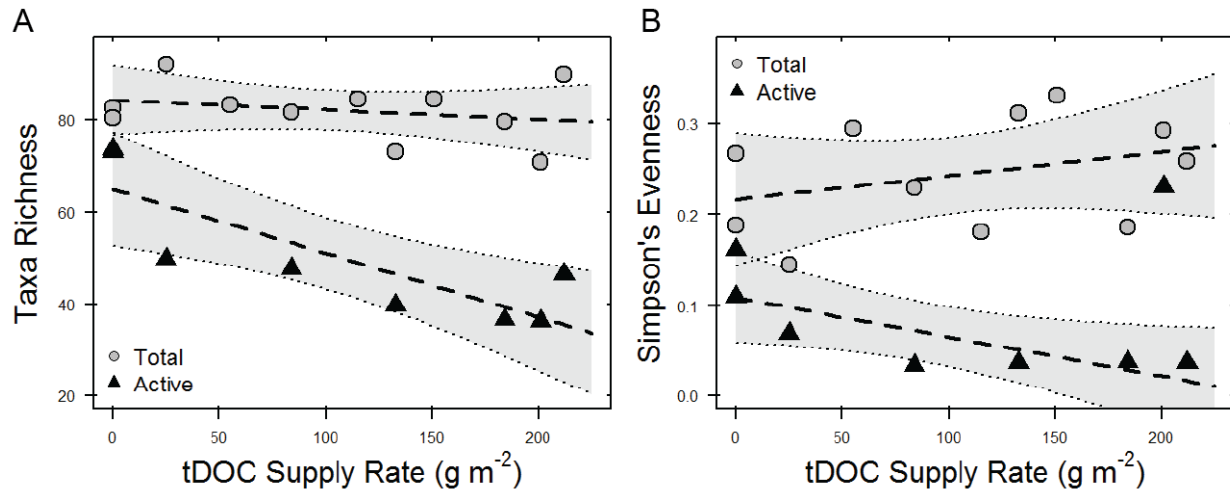
606 indicates a more stable community. Based on the total community (DNA, circles),

responsiveness did not change in as tDOC supply rate increased ( $P = 0.37$ ). However, based on  
608 the active community (RNA, triangles), responsiveness decreased as tDOC supply rate increased  
( $P < 0.001$ ).

610

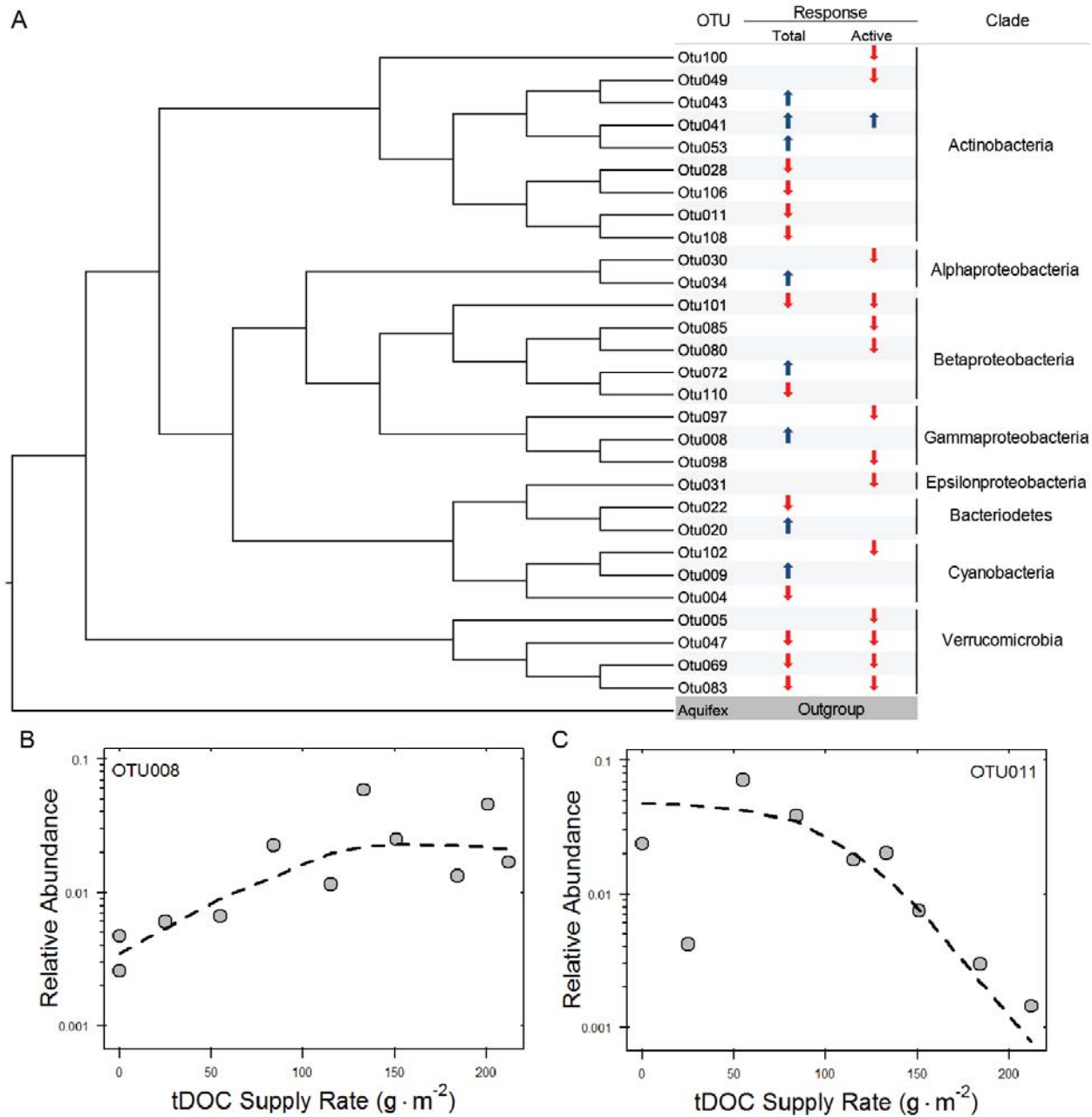
## FIGURES

612 **Figure 1:**



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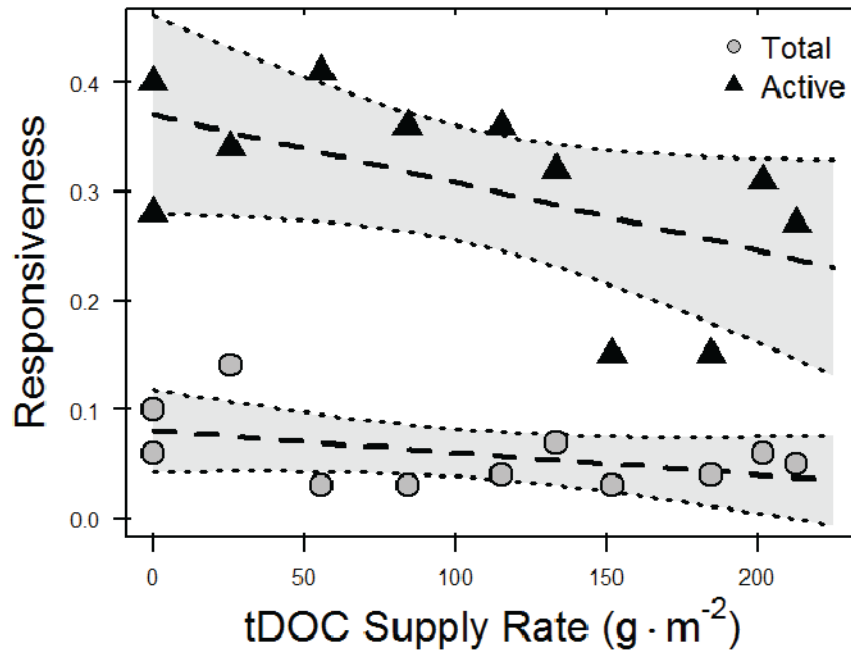
**Figure 2:**



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**Figure 3:**



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