

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

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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 (NH_4NO_3 and Na_2HPO_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 µm Supor PES membrane filters (Pall) and stored immediately at -80 °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,
156 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
158 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
160 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
162 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
164 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
166 (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
168 statistical calculations were performed in the R computing environment (v 3.1.3, R Core
Development Team 2012).

170

Community Composition and Species Sorting – To determine the effects of tDOC supply on
172 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/m²) 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**

In addition to changes in diversity, the altered resource environment modified the
290 composition of aquatic bacterial communities by selecting for and against certain taxa (i.e.
species sorting). We observed significant increases and decreases in taxa along the resource
292 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
294 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
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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418 analyses is available at <https://github.com/LennonLab/SubsidyMicrobialStability>.

REFERENCES

- 420 Aanderud, Z., S. Jones, N. Fierer, J. T. Lennon, Z. T. Aanderud, S. Jones, N. Fierer, and J. T.
422 Lennon. 2015. Resuscitation of the rare biosphere contributes to pulses of ecosystem
activity. *Frontiers in Microbiology* 6:1–11.
- 424 Allison, S. D., and J. B. H. Martiny. 2008. Resistance, resilience, and redundancy in microbial
communities. *Proceedings of the National Academy of Sciences* 105:11512–11519.
- 426 Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance.
Austral Ecology 26:32–46.
- 428 Anderson, M. J., K. E. Ellingsen, and B. H. McArdle. 2006. Multivariate dispersion as a measure
of beta diversity. *Ecology Letters* 9:683–93.
- 430 APHA. 1998. *Standard Methods for the Examination of Water and Wastewater*. (L. S. Clesceri,
A. D. Eaton, and A. E. Greenberg, Eds.). 20th edition. American Public Health Association,
432 Washington, D.C.
- Bartels, P., J. Cucherousset, K. Steger, P. Eklöv, L. J. Tranvik, and H. Hillebrand. 2012.
434 Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer
resource dynamics. *Ecology* 93:1173–82.
- 436 Bastviken, D. 2004. Methane emissions from lakes: Dependence of lake characteristics, two
regional assessments, and a global estimate. *Global Biogeochemical Cycles* 18:1–12.
- 438 Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate: A Practical and
Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society* 57:289 –
440 300.
- Brown, B. L. 2003. Spatial heterogeneity reduces temporal variability in stream insect
442 communities. *Ecology Letters* 6:316–325.
- Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. S. Kulam-Syed-Mohideen, D.
444 M. McGarrell, T. Marsh, G. M. Garrity, and J. M. Tiedje. 2009. The Ribosomal Database
Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Research*
446 37:D141–D145.
- Comte, J., L. Fauteux, and P. A. del Giorgio. 2013. Links between metabolic plasticity and
448 functional redundancy in freshwater bacterioplankton communities. *Frontiers in
Microbiology* 4:1–11.
- 450 Comte, J., and P. A. del Giorgio. 2011. Composition Influences the Pathway but not the
Outcome of the Metabolic Response of Bacterioplankton to Resource Shifts. *PLoS ONE*
452 6:e25266.

- 454 Cottingham, K. L., and S. R. Carpenter. 1994. Predictive Indexes of Ecosystem Resilience in
Models of North Temperate Lakes. *Ecology* 75:2127–2138.
- 456 Cottingham, K., and L. Narayan. 2013. Subsidy quantity and recipient community structure
mediate plankton responses to autumn leaf drop. *Ecosphere* 4:89.
- 458 DeAngelis, D. L. 1992. *Dynamics of Nutrient Cycling and Food Webs*. Chapman and Hall,
London, UK.
- 460 Elser, J. J., K. Acharya, M. Kyle, J. Cotner, W. Makino, T. Markow, T. Watts, S. Hobbie, W.
Fagan, J. Schade, J. Hood, and R. W. Sterner. 2003. Growth rate-stoichiometry couplings in
diverse biota. *Ecology Letters* 6:936–943.
- 462 Elser, J., E. R. Marzolf, and C. R. Goldrnan. 1990. Phosphorus and Nitrogen Limitation of
Phytoplankton Growth in the Freshwaters of North America: A Review and Critique of
464 Experiments Enrichments. *Canadian Journal of Botany* 47:1468–1477.
- 466 Faria, L. D. B., and M. I. D. S. Costa. 2010. Omnivorous food web, prey preference and
allochthonous nutrient input. *Ecological Complexity* 7:107–114.
- 468 Fellman, J. B., M. P. Miller, R. M. Cory, D. V D’Amore, and D. White. 2009. Characterizing
dissolved organic matter using PARAFAC modeling of fluorescence spectroscopy: a
comparison of two models. *Environmental Science & Technology* 43:6228–34.
- 470 Fierer, N., M. Hamady, C. L. Lauber, and R. Knight. 2008. The influence of sex, handedness,
and washing on the diversity of hand surface bacteria. *Proceedings of the National
472 Academy of Sciences* 105:17994–9.
- 474 Fuchs, G., M. Boll, and J. Heider. 2011. Microbial degradation of aromatic compounds - from
one strategy to four. *Nature Reviews Microbiology* 9:803–816.
- 476 del Giorgio, P. A., and J. M. Gasol. 2008. Physiological structure and single-cell activity in
marine bacterioplankton. Pages 243–298 in D. L. Kirchman, editor. *Microbial Ecology of
the Oceans*. Second edition. John Wiley & Sons, Inc., Hoboken, NJ, USA.
- 478 Godwin, C. M., and J. B. Cotner. 2015. Aquatic heterotrophic bacteria have highly flexible
phosphorus content and biomass stoichiometry. *The ISME Journal* 9:2324–2327.
- 480 Grimm, V., and C. Wissel. 1997. Babel, or the ecological stability discussions: An inventory and
analysis of terminology and a guide for avoiding confusion. *Oecologia* 109:323–334.
- 482 He, X., M. D. Scheurell, P. A. Soranno, and R. A. Wright. 1994. Recurrent response patterns of a
zooplankton community to whole-lake fish manipulation. *Freshwater Biology* 32:61–72.
- 484 Holt, R. D. 2004. Implications of System Openness for Local Community Structure and
Ecosystem Function. Pages 96–114 in G. A. Polis, M. E. Power, and G. R. Huxel, editors.

- 486 Food Webs at the Landscape Level. University of Chicago Press, Chicago.
- Huxel, G. R., and K. McCann. 1998. Food web stability: the influence of trophic flows across
488 habitats. *The American Naturalist* 152:460–9.
- Jablonski, D. 2008. Species Selection: Theory and Data. *Annual Review of Ecology, Evolution,
490 and Systematics* 39:501–524.
- Jones, S. E., and J. T. Lennon. 2010. Dormancy contributes to the maintenance of microbial
492 diversity. *Proceedings of the National Academy of Sciences* 107:5881–6.
- Jones, S. E., and J. T. Lennon. 2015. A test of the subsidy – stability hypothesis: the effects of
494 terrestrial carbon in aquatic ecosystems. *Ecology* 96:1550–1560.
- Jones, S. E., R. J. Newton, and K. D. McMahon. 2009. Evidence for structuring of bacterial
496 community composition by organic carbon source in temperate lakes. *Environmental
Microbiology* 11:2463–72.
- Judd, K. E., B. C. Crump, and G. W. Kling. 2006. Variation in dissolved organic matter controls
498 bacterial production and community composition. *Ecology* 87:2068–79.
- 500 Karlsson, J., P. Byström, J. Ask, P. Ask, L. Persson, and M. Jansson. 2009. Light limitation of
nutrient-poor lake ecosystems. *Nature* 460:506–509.
- 502 Kritzberg, E. S., J. J. Cole, M. L. Pace, W. Granéli, and D. L. Bade. 2004. Autochthonous versus
allochthonous carbon sources to bacteria: Results from whole-lake ¹³C addition
504 experiments. *Limnology and Oceanography* 49:588–596.
- Lapierre, J.-F., F. Guillemette, M. Berggren, and P. A. Del Giorgio. 2013. Increases in
506 terrestrially derived carbon stimulate organic carbon processing and CO₂ emissions in
boreal aquatic ecosystems. *Nature Communications* 4:2972.
- 508 Legendre, P., and L. F. J. Legendre. 2012. *Numerical ecology*. Elsevier.
- Lennon, J. T., A. M. Faiia, X. Feng, and K. L. Cottingham. 2006. Relative importance of CO₂
510 recycling and CH₄ pathways in lake food webs along a dissolved organic carbon gradient.
Limnology and Oceanography 51:1602–1613.
- 512 Lennon, J. T., S. K. Hamilton, M. E. Muscarella, A. S. Grandy, K. Wickings, and S. E. Jones.
2013. A source of terrestrial organic carbon to investigate the browning of aquatic
514 ecosystems. *PLOS ONE*. *PLoS One* 8:e75771.
- Lennon, J. T., and S. E. Jones. 2011. Microbial seed banks: the ecological and evolutionary
516 implications of dormancy. *Nature Reviews Microbiology* 9:119–30.
- Livermore, J. A., S. J. Emrich, J. Tan, and S. E. Jones. 2014. Freshwater bacterial lifestyles
518 inferred from comparative genomics. *Environmental Microbiology* 16:746–58.

- Monteith, D., J. Stoddard, C. Evans, H. A. de Wit, M. Forsius, T. Hogasten, A. Wilander, B. L.
520 Skjelkvåle, D. S. Jeffries, J. Vuoremnaa, B. Keller, J. Kopáček, and J. Vesely. 2007.
522 Dissolved organic carbon trends resulting from changes in atmospheric deposition
chemistry. *Nature* 450:537–540.
- Mou, X., S. Sun, R. A. Edwards, R. E. Hodson, and M. A. Moran. 2008. Bacterial carbon
524 processing by generalist species in the coastal ocean. *Nature* 451:708–11.
- Muscarella, M. E., K. Bird, M. L. Larse, S. A. Placella, and J. T. Lennon. 2014. Phosphorus
526 resource heterogeneity in microbial food webs. *Aquatic Microbial Ecology* 73:259–272.
- Nelson, C. E., and C. A. Carlson. 2012. Tracking differential incorporation of dissolved organic
528 carbon types among diverse lineages of Sargasso Sea bacterioplankton. *Environmental
Microbiology* 14:1500–16.
- 530 Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. *Applied linear statistical
models*. Irwin, Chicago, Illinois, USA.
- 532 Newton, R. J., L. E. Griffin, K. M. Bowles, C. Meile, S. Gifford, C. E. Givens, E. C. Howard, E.
King, C. A. Oakley, C. R. Reisch, J. M. Rinta-Kanto, S. Sharma, S. Sun, V. Varaljay, M.
534 Vila-Costa, J. R. Westrich, and M. A. Moran. 2010. Genome characteristics of a generalist
marine bacterial lineage. *The ISME Journal* 4:784–798.
- 536 Newton, R. J., S. E. Jones, A. Eiler, K. D. McMahon, and S. Bertilsson. 2011. A guide to the
natural history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews*
538 75:14–49.
- Nowlin, W. H., M. J. González, M. J. Vanni, M. H. H. Stevens, M. W. Fields, and J. J. Valente.
540 2007. Allochthonous subsidy of periodical cicadas affects the dynamics and stability of
pond communities. *Ecology* 88:2174–86.
- 542 Nowlin, W. H., M. J. Vanni, and L. H. Yang. 2008. Comparing Resource Pulses in Aquatic and
Terrestrial Ecosystems. *Ecology* 89:647–659.
- 544 Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O’Hara, G. L. Simpson,
P. Solymos, M. H. H. Stevens, and H. Wagner. 2013. *Vegan: community ecology package*.
- 546 Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food
web ecology: the dynamics of spatially subsidized food webs. *Annual Review of Ecology
548 and Systematics* 28:289–316.
- R Core Development Team. 2012. *R: A language and environment for statistical computing*.
- 550 Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A.
Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G.
552 Thallinger, D. J. Van Horn, and C. F. Weber. 2009. Introducing mothur: open-source,

- 554 platform-independent, community-supported software for describing and comparing
microbial communities. *Applied and Environmental Microbiology* 75:7537–7541.
- Shade, A., J. S. Read, D. G. Welkie, T. K. Kratz, C. H. Wu, and K. D. McMahon. 2011.
556 Resistance, resilience and recovery: aquatic bacterial dynamics after water column
disturbance. *Environmental Microbiology* 13:2752–67.
- 558 Smith, B., and J. B. Wilson. 1996. A consumer’s guide to evenness indices. *Oikos* 76:70–82.
- Solomon, C. T., S. E. Jones, B. C. Weidel, I. Buffam, M. L. Fork, J. Karlsson, S. Larsen, J. T.
560 Lennon, J. S. Read, S. Sadro, and J. E. Saros. 2015. Ecosystem Consequences of Changing
Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future
562 Challenges. *Ecosystems* 18:376–389.
- Takimoto, G. 2002. Seasonal subsidy stabilizes food web. *Ecological Research* 17:433–439.
- 564 Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular
Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30:2725–
566 2729.
- Tilman, D. 1977. Resource competition between plankton algae: an experimental and theoretical
568 approach. *Ecology* 58:338–348.
- Tranvik, L., J. Downing, and J. Cotner. 2009. Lakes and reservoirs as regulators of carbon
570 cycling and climate. *Limnology and Oceanography* 54:2298–2314.
- Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian Classifier for Rapid
572 Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Applied and
Environmental Microbiology* 73:5261–5267.
- 574 Wetzel, R. G. 1999. Biodiversity and shifting energy stability within freshwater ecosystems.
Arch Hydrobiol Spec Issues Advanc Limnol 54:19–32.
- 576 Wetzel, R. G., and G. E. Likens. 2000. Inorganic nutrients: nitrogen, phosphorus, and other
nutrients. Pages 85–111 *Limnological Analyses*. Third edition. Springer-Verlag, New York.
- 578 Wilkinson, G. M., M. L. Pace, and J. J. Cole. 2013. Terrestrial dominance of organic matter in
north temperate lakes. *Global Biogeochemical Cycles* 27:43–51.
- 580 Wilson, D. S., and J. Yoshimura. 1994. On the coexistence of specialists and generalists. *The
American Naturalist* 144:692–707.
- 582

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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

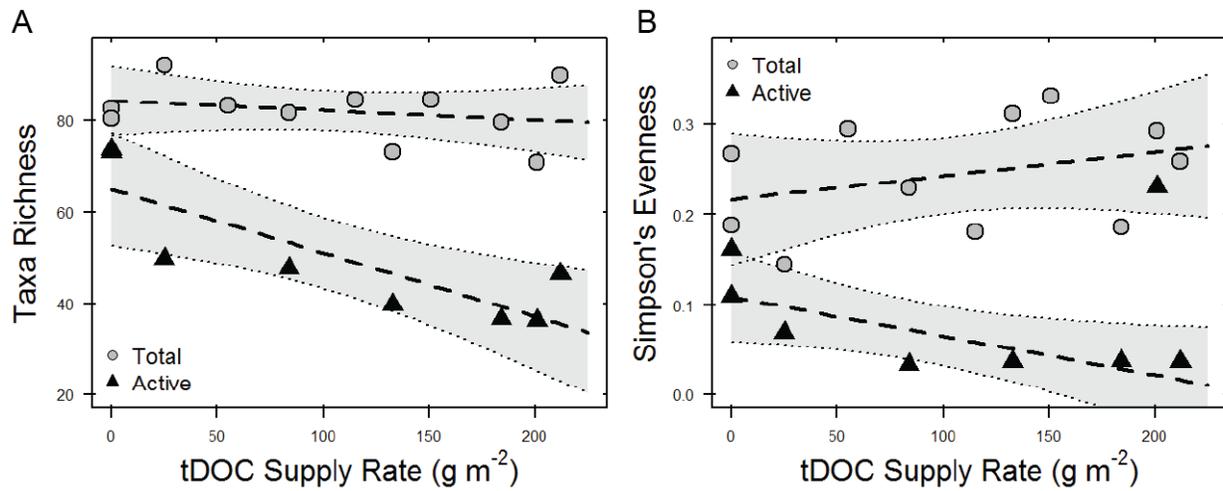
606 indicates a more stable community. Based on the total community (DNA, circles),

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
($P < 0.001$).

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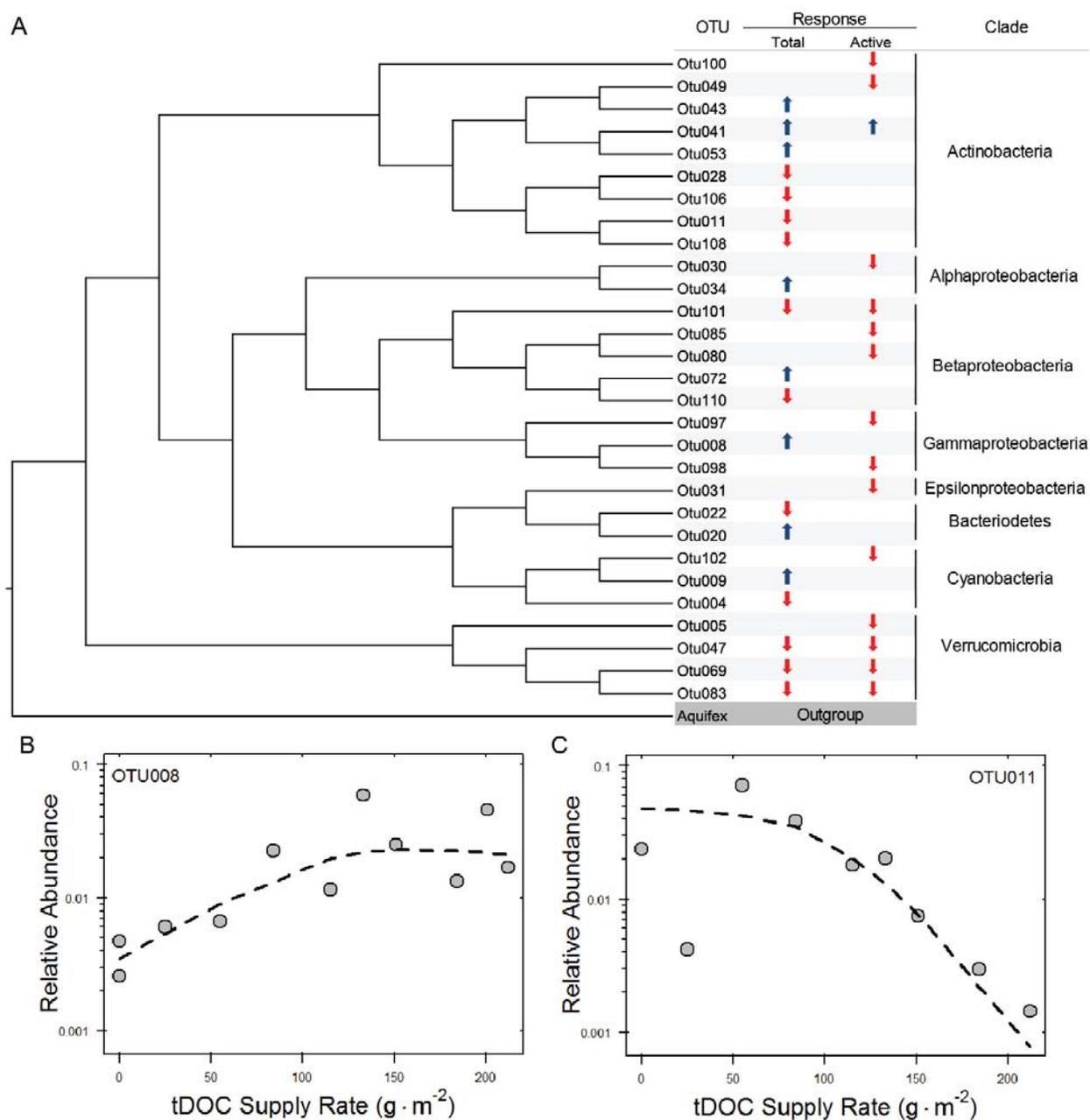
FIGURES

612 **Figure 1:**



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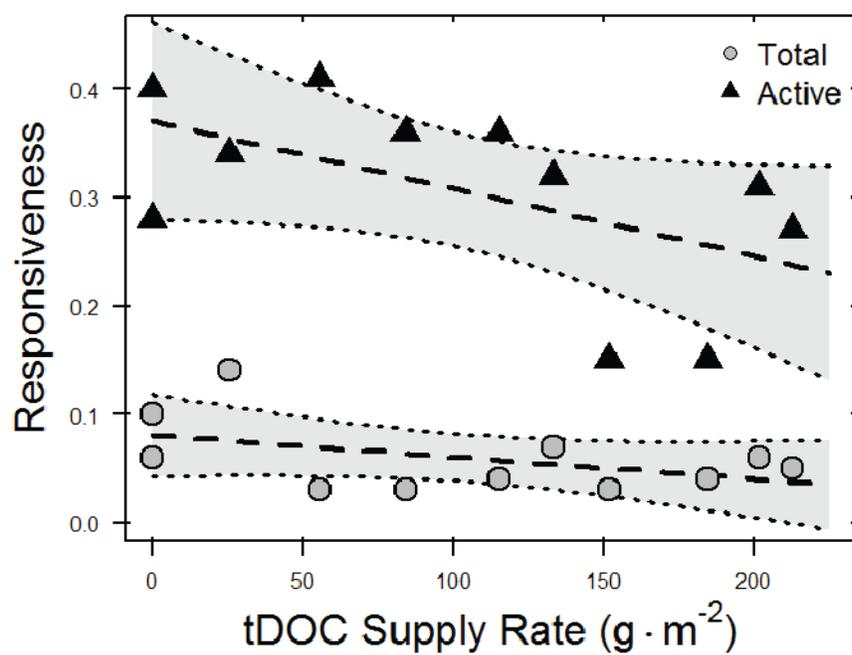
Figure 2:



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Figure 3:



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