Time-variant species pools shape competitive dynamics and biodiversity-ecosystem function relationships

Author: David W. Armitage [a, b]

Affiliations:

[a] Department of Integrative Biology, University of California Berkeley

3040 Valley Life Sciences Building

Berkeley, CA, USA 94720-3140

[b] Department of Biological Sciences, University of Notre Dame

100 Galvin Life Science Center

Notre Dame, IN, USA 46556

Correspondence:

David W. Armitage

Department of Biological Sciences, University of Notre Dame

100 Galvin Life Science Center

Notre Dame, IN, USA 46556

E-mail: dave.armitage@gmail.com

Tel: (248)-736-4174

Keywords: bacteria, biodiversity-ecosystem function, competition, pitcher plant, succession, time

Abstract

Biodiversity-ecosystem function (BEF) experiments routinely employ common garden designs, drawing samples from a local biota. The communities from which taxa are sampled may not, however, be at equilibrium. To test for temporal changes in BEF relationships, I assembled pools of aquatic bacterial strains isolated at different time points from leaves on the pitcher plant *Darlingtonia californica* in order to evaluate the strength, direction, and drivers of the BEF relationship across a natural host-associated successional gradient. I constructed experimental communities using bacterial isolates from each time point and measured their respiration rates and competitive interactions. Communities assembled from mid-successional species pools showed the strongest positive relationships between community richness and respiration rates, driven primarily by linear additivity among isolates. Diffuse competition was common among all communities but greatest within mid-successional isolates. These results demonstrate the dependence of the BEF relationship on the temporal dynamics of the local species pool, implying that ecosystems may respond differently to the addition or removal of taxa at different points in time during succession.

INTRODUCTION

The rates at which ecosystems cycle nutrients are predicted to be set in predominantly by the actions of their constituent organisms [1–3]. Over the past two decades, this conceptual unification of communities and ecosystems has been empirically evaluated using the biodiversity - ecosystem function (BEF) framework [4–6]. This research commonly reports a positive covariance between species richness and community biomass production and is hypothesized to be jointly driven by community members' differential contributions to ecosystem properties (selection effects) and their degree of niche overlap (complementarity effects) [7].

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

The relative importance of these effects is in large part a function of resource competition among community members [8]. Many ecosystem functions are enabled by a single guild of competitors. If taxa within a guild vary in their contributions to ecosystem function, then turnover resulting from interspecific competition should result in shifting BEF relationships. Communities, however, are naturally dynamic and can experience both gradual successional turnover and rapid state transitions [9,10]. Such turnover is predicted to result, in part, from temporal variation in species interactions — particularly competition — as new taxa arrive and changing local conditions lead to fitness differences among competitors [11]. Because the strength of resource competition among community members is predicted to vary over the course of primary succession [1,12,13] and also influence the magnitude and drivers of the BEF relationship, it stands that the BEF relationship should vary along a successional gradient. Thus, a comprehensive theory linking biodiversity to ecosystem function must explicitly account for the effects of community turnover through time [14,15].

The majority of BEF experiments track the productivity of monocultures and polycultures assembled from taxa randomly drawn from a natural biota or from *ad hoc* combinations of tractable organisms such as algae or protists. In these experimental communities, the magnitude and drivers of the BEF relationship are often found to change over time [16–26]. While these experiments have contributed fundamental insights into the temporal dynamics of BEF relationships, they do not account for a dynamic species pool. In other words, the groups of species used to seed these communities represent either a *snapshot* of a natural community at a particular point in time (fig. 1A) or a collection of species that may be differentially distributed across time such that two species added into a community do not necessarily co-occur under natural settings (fig. 1B). Communities assembled from a

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

dynamic species pool, however, may show different BEF relationships over time due to the shifting identities and interactions of the constituent taxa (fig. 1C).

Whereas biodiversity-ecosystem function experiments are most commonly conducted using primary producers, the framework has also been successfully extended to other groups. In particular, bacterial communities have been the subject of numerous BEF studies, owning to both their experimental tractability and importance in regulating ecosystem processes [18,24,27–30]. Because natural bacterial communities often exhibit marked turnover through time [31,32] they provide an opportunity to investigate the strength and drivers of the BEF relationship over a temporal gradient.

Carnivorous pitcher plants in the family Sarraceniaceae are a group for which bacterial communities provide a particularly critical function. These plants have evolved to capture arthropod prey by means of a conical leaf in which trapped insects are drowned by fluid secreted by the host [33,34]. Digestion is facilitated both by enzymes produced by the plant and by a dynamic community of bacteria residing in the fluid [32,35–37]. The pitcher plant *Darlingtonia californica* (Torr.) is hypothesized to rely heavily on bacteria for prey digestion [35]. The pitcher leaves of this species are produced at regular intervals throughout the June-October growing season and are sterile prior to opening [32]. Once the leaves fully develop, they quickly begin trapping insects, and bacterial biomass skyrockets to over 10^9 cells mL^{-1} [32]. After approximately two months, a leaf ceases prey capture but remains photosynthetically active for a second growing season. Bacterial diversity in *Darlingtonia* pitchers changes predictably over time, as has been documented by both culture-independent molecular approaches as well as among bacterial cultures isolated from different aged leaves [32,38]. These temporal isolates provide a unique opportunity to experimentally test the

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

relationship between biodiversity and ecosystem functioning along a natural microbial successional gradient.

My goals for this study were twofold. First, I investigated whether the contribution of bacterial richness to rates of carbon mineralization changed over time along a natural successional gradient in *Darlingtonia* leaves. Second, I used these data to estimate the relative influences of individual strains and their interspecific interactions (such as competition) on the BEF relationship [39]. The strength of interspecific competition among bacterial strains growing in a polyculture can be approximated as the difference between the community's predicted respiration in the absence of any interference (i.e., the sum of the strains' monoculture respiration rates) and the community's realized respiration rate, given the mono- and polycultures have equal total starting densities [40,41]. If strains in a polyculture do not inhibit one another through resource competition or direct antagonism, then the community's rate of carbon respiration will not significantly differ from the additive monoculture expectation [40]. This measure of competitive inhibition is anticipated to increase over time if, for instance, a competition-colonization tradeoff results in the dominance of early pitcher leaves by less-competitive, ruderal taxa which are later excluded by superior competitors [11,13]. Alternatively, the bacterial taxa dominating late-stage pitchers may be specialists on recalcitrant carbon resources and therefore may not contribute significantly to respiration, compared to early, fast-growing colonists [42]. In this case, I anticipated a negative trend in competitive inhibition over time. In order to experimentally test these hypotheses, I assembled synthetic microbial communities using pools of bacterial strains isolated from a cohort of pitcher leaves at regular intervals and measured their rates of carbon mineralization.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

MATERIAL AND METHODS

Sample collection & strain isolation

In the field, I tagged five unopened *Darlingtonia* pitcher leaves of the same approximate age at the beginning of the growing season and tracked them over their first year. I visited this cohort of leaves every 11 days from June to September 2014 and once in June 2015 to remove 0.5 mL of pitcher fluid from each leaf. This fluid was diluted and spread on R2A agar plates and incubated at 25° C, and bacterial colonies expressing unique colony morphologies, cellular morphologies, and pigmentations were isolated in pure culture. The 10 most abundant bacterial strains isolated from each pitcher age class were then used to inoculate experimental microcosms (table S1). Extended discussion of the sampling and isolation methods can be found in the electronic supplementary materials accompanying this article. Supplementary figure S1 provides a graphical walkthrough of the experimental procedure.

Microcosm experiment

I combined the 10 strains isolated from each time point into 1-, 2-, 5-, and 10-strain communities using the random partitions design introduced by Bell *et al*. [39]. My experiment consisted of 4 partitions (P), each containing 4 strain richness treatments (R) and $10/R$ randomized communities within each $P \times R$ treatment (supplemental fig. S2). Every experimental community was replicated 3 times. This experimental design ensures that all species are equally represented within and among richness levels, giving each one an equal opportunity to contribute to selection and complementarity effects and weakens statistical artifacts such as the 'variance reduction effect' [43]. It also permits the statistical separation of species effects and richness effects on ecosystem processes without the need for measuring the contribution of an individual species to the properties of the polyculture, as is

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

151 traditional in BEF studies using plant biomass as a response. This enables the user to estimate
152 species' contributions to emergent ecosystem properties (e.g. carbon mineralization rates)
153 that cannot be attributed to individual taxa in polyculture. Furthermore, it relaxes the
154 requirement for a full-factorial experimental design, which becomes intractable as the
155 number of species increases. In total, I assembled 216 communities per time point, resulting
156 in a total of 1944 cultures spanning 9 source community ages and 4 levels of richness.
157
158 The bacterial microcosms consisted of 1.2 mL 96 well plates containing a sterile
159 artificial pitcher medium comprised of M9 salt solution and ground cricket powder.
160 Individual bacterial strains were grown to mid-log-phase in R2A broth, washed of their
161 medium, and starved for two hours. Each strain was introduced into its community at the
162 volume required to keep the total number of cells across richness treatments equal (100 µL,
163 or approximately 10^4 colony forming units). Once assembled, plates were clamped onto 96-
164 well MicroResp™ (James Hutton Institute, Inc.) respirometry plates containing a
165 colorimetric CO_2 indicator solution [44]. All replicate communities for a single time point
166 were incubated simultaneously at 25° C for three days, after which time I estimated rates of
167 CO_2-C entering each agar well on the MicroResp™ plate from its absorbance at 590 nm on a
168 microplate reader. I measured the carbon metabolic profiles of each 10-strain community
169 using the GN2 microplate (Biolog, Inc.), which assays a community's potential to metabolize
170 95 different carbon compounds. Each Biolog assay was run in triplicate at 25° C for three
171 days and only substrates scoring positive for metabolism across all replicates were scored as
172 positive. I used ANOVA to test for differences in the mean number of compounds used
173 between community ages and principal coordinates analysis to ordinate samples' metabolic
174 profiles based on their Jaccard distances. Additional experimental procedures are detailed in
175 the electronic supplemental materials.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

176

177 *Statistical analyses*

178 To assess how drivers of the BEF relationship differed among time points, I fit a linear model

179 to community respiration rates [39]. This model took the form

$$y = \beta_0 + \beta_{LR}x_{LR} + \beta_{NLR}x_{NLR} + \left(\sum_i^S \beta_i x_i\right) + \beta_Q x_Q + \beta_M x_M + \varepsilon \quad (1)$$

180 where y is a community or ecosystem process (e.g., respiration rate), β_{LR} is the effect of strain

181 richness measured on a continuous scale (linear richness, x_{LR}), β_{NLR} is the effect of strain

182 richness measured on a categorical scale (non-linear richness, x_{NLR}), β_i is the impact of an

183 individual strain's presence on the productivity of its community, β_Q is the effect of the

184 particular taxon pool used in each $P \times R$ treatment, and β_M is the effect of a particular

185 community composition within each taxon pool, β_0 is the intercept, and ε is the error term.

186

187 Importantly, by estimating the linear richness term prior to the nonlinear richness and

188 strains' impact terms, the latter two terms become orthogonal. The species impact (β_i) terms

189 sum to zero and reflect the relative influence an individual strain exerts on the community's

190 respiration. The nonlinear richness term (β_{NLR}) can be interpreted as the magnitude of

191 deviations from linear richness effects. Nonzero values of β_{NLR} reflect the influence of

192 facilitative and competitive interactions on ecosystem processes. I used least squares to

193 estimate the model coefficients and an F-test to determine the statistical significance of each

194 variable. The denominator term for the F-statistics of the β_{NLR} and β_i parameters were the

195 partitioned mean squares from the species pool (Q) or species composition (M) factors,

196 respectively. Model terms were entered in the order in which they appear in equation 2:

197 nonlinear richness (β_{NLR}) and species impacts (β_i) were estimated from the residuals of the

198 model containing the linear richness (β_{LR}) term.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

I estimated the effects of the source pitchers' ages and experimental communities' richness on rates of CO_2 respiration using linear regression. To aid in the interpretation of interactions, predictors were centered to their mean values prior to model fitting. I assessed the pairwise differences among community ages using Tukey's range test ($\alpha = 0.05$). Community age was treated as an ordinal, discrete variable to account for the absence of sampling between days 88 and 365.

I estimated the extent to which strains inhibit one another's potential CO_2 production in polyculture by calculating the difference between a community's predicted and observed respiration rates. The predicted values were calculated by summing all community members' average monoculture respiration rates. The difference between a polyculture's predicted and observed respiration values will equal zero if there are no inhibitory effects between members of the community (i.e., all taxa in a polyculture perform as well they do in monoculture) [40,41]. Alternatively, direct antagonism (e.g., antibiotic production) or resource competition is anticipated to result in respiration rates less than the additive prediction. I used ANCOVA to test the null hypothesis that the mean differences between predicted and observed respiration rates were equal among community ages, controlling for richness effects. Pairwise differences between centered predictor variables were assessed using Tukey's range test. All models were fit using R v3.1 [45].

Pairwise antagonism assay

I performed spot assays to determine whether a particular bacterial strain directly inhibits the growth of a co-occurring strain. I created lawns of focal strains by spreading log-phase broth cultures onto two plates containing R2A agar onto which I spotted 2 µL log-phase broth

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

culture of each co-occurring isolate. Each spot was replicated four times on the same plate, resulting in 8 cross-inoculations per strain pair (excluding sterile blanks). After 24 hours at 25° C, I searched for zones of clearing surrounding a colony. I considered the spotted strain to be inhibitory to the focal strain if unambiguous zones of clearing surrounded at least 6 replicates.

RESULTS

I found an average of 6.9 (SE = 0.18, range = 5-9) bacterial strains remaining in each 10-strain community, and there were no significant differences in the proportions of surviving strains among source community ages ($F_{8,27} = 2.3$, $p = 0.06$). Thus, although the strains' relative abundances changed throughout the incubation period, no single, dominant strain was able to exclude the majority of others. I detected significant differences between the mean respiration rates of bacterial communities isolated from pitcher leaves of different ages (table 1, fig. S3). Post-hoc analysis revealed respiration rates to be greatest among bacterial communities isolated from pitcher leaves between 22 and 55 days old (fig. S3). This pattern was consistent under all four richness treatments, although there was a general tendency for variance in respiration rates among treatments to increase when more strains were present. Bacterial richness had a significantly positive effect on overall respiration rates, ($\beta_R = 0.05 \pm 0.007$; table 1, fig. 2), although there was a significant interaction between richness and source community age (table 1).

The effect of linear richness (β_{LR}) on respiration rates was significantly positive for all source community ages except those from days 88 and 365 (fig. 2). This positive effect of richness on respiration was greatest for isolates from pitcher leaves between 22 and 66 days old, and tended to increase from days 11 to 22 and then slowly decrease towards zero

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

throughout the rest of the pitchers' lifespan (fig. 3A). For each bacterial isolate pool, I detected individual nonlinear richness effects (β_{NLR}) and individual strain (β_i) effects significantly greater or less than zero, but these effects were not significant overall (table S2). Despite this, the relative influence of nonlinear richness effects was greater than overall strain effects for the majority of time points (fig. 3B).

The average differences between expected and observed respiration rates initially increased between samples collected from 11-day and 22-day pitchers, and then declined with source community age (fig. 4). There were a number of instances where the observed respiration rates of two-strain mixtures were greater than their predicted values, but overall mean values were significantly greater than zero for all richness treatments ($\beta_{0,R2} = 1.04 \pm 0.26$, $\beta_{0,R5} = 7.25 \pm 0.48$, $\beta_{0,R10} = 16.05 \pm 0.62$, $p < 0.0001$ for all cases). The magnitude of this inhibitory effect increased with strain richness (fig. 4, table 1). I only detected 12 antagonistic interactions between eight pairs of strains (out of 405 total). These interactions occurred only in 11- and 44-day source pools. Furthermore, there was no detectable temporal trend among source pool ages in either the total number of carbon substrates utilized (fig. S4) or their multivariate Jaccard similarities (fig. S4).

DISCUSSION

Dynamic species pools impact BEF relationships

I encountered a mid-successional peak in rates of carbon mineralization, independent of taxonomic richness. This implies that when placed into identical environments, bacterial strains isolated from leaves of intermediate ages (22 to 55 days old) were better able to mineralize carbon in the growth medium. This result could not be explained by differences in the taxon pools' carbon metabolic profiles. Rather, the increase in strains' average respiration

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

rates during this period coincided with the greatest rates of prey capture by the pitcher leaf [32,46]. It is possible that the relatively low respiration rates of late-stage bacterial communities reflect an adaptive strategy for living in nutrient-poor pitcher environments. This is supported by the observation of lower average ribosomal RNA copy numbers — a trait correlated with growth rate— as succession proceeds [32,47]. However, information on all strains' relative performances across different nutrient concentrations would be required to experimentally verify this hypothesis. A recent study found that both BEF effects and competitive interactions decreased in bacterial microcosms over time, as highly-productive taxa were outcompeted by specialists capable of efficient use of recalcitrant resources [42]. This observation mirrors and lends support to my results, and suggests that the community dynamics observed in closed microcosms may approximate those from more natural systems.

The effects of a community's richness on respiration rates were generally positive, but varied over time such that the slope estimates peaked in pitcher leaves of intermediate age. These positive BEF relationships appeared to be driven by linear, additive contributions of taxa, as evidenced by strong positive linear richness terms, but weak nonlinear richness and species impact terms. This observation implies that, on average, community members had similar relative respiration rates and low levels of niche overlap. This interpretation is supported by the lack of dominance by any one or more strains in 10-strain communities, which would be predicted to lead to significant species impact terms. Inhibition of a community's potential additive respiration rates was common in all polycultures and peaked in communities assembled from intermediate-aged pitcher leaves. This observation, combined with an absence of direct antagonistic interactions, provides evidence for diffuse competition limiting a strain's potential respiration in polyculture. Although I failed to detect significant negative nonlinear richness terms indicative of strong competition, I did encounter

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

299 an increase in the effect of nonlinear richness coinciding with the periods of highest
300 respiration inhibition. This general pattern of diffuse competition in polycultures is
301 commonly found in bacterial microcosm experiments [40,48] but may not be typical of
302 bacteria within pitcher plants due to my isolation procedure. By using a single medium to
303 isolate bacteria, it is likely that the strains I sampled were more phenotypically similar to one
304 another than to a random sample of all bacteria in a pitcher leaf. Thus, the strains used in this
305 study should be considered members sampled from a guild of aerobic, heterotrophic bacteria
306 and are expected to compete with one another for resources and express similar rates of
307 carbon respiration. However, this is no different than most plant and microbial BEF studies,
308 which commonly draw inference at the guild level. A useful follow-up to this experiment
309 would investigate the effects of increasing the phenotypic diversity of the taxon pool by
310 adding strains obtained using a broader range of media.
311
312 Competition among isolates is predicted to decrease in bacterial communities over
313 time due to divergent evolution and can lead to changes in ecosystem functioning [49–51].
314 The relatively low levels of competitive inhibition among strains from late-stage pitcher
315 leaves may represent indirect evidence of divergence. This scenario is plausible, given the
316 rapid generation times and population sizes of the isolates. A recent study by Fiegna *et al*.
317 [48] showed that the experimental evolution of bacterial isolates over 5 weeks can alter the
318 BEF relationship via a relaxation of competition. Although such an effect is possible in
319 natural systems, its demonstration would require tracking individual bacterial lineages over
320 time and regularly assaying their competitive interactions. Miller and Kneitel [52] attempted
321 this by measuring the degree of competitive inhibition of four bacterial colony morphotypes
322 isolated from the same pitcher leaves 7 days and 42 days after opening. The authors found
323 that the competitive abilities (relative to a common bacterial competitor) of two of the four

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

strains decreased with pitcher age while two did not appear to change [52]. These results match my observation of increased competitive inhibition of potential respiration on a similar timescale (11-day and 44-day leaves).

Potential drivers of BEF relationships

To date, few studies have directly estimated the impacts of natural successional dynamics in the context of biodiversity and ecosystem functioning [26,53,54]. Using 15 years of observational data from regenerating tropical forest plots, Lasky *et al.* documented a decreasing effect of species richness on rates of aboveground biomass production in mid- and late-successional tropical forest plots [26]. These results matched both theoretical predictions [14] and experimental studies in which diversity effects were tracked over time within individual microcosms without immigration [18,22]. My results conform to those of other BEF time-series experiments, despite marked differences in design. In concert, these findings challenge the common observation that the effects of richness on productivity become more positive over time [21], though further investigation is necessary to uncover the mechanisms leading to these contrasting outcomes.

One mechanism for generating non-positive BEF relationships is the negative selection effect [7,27]. This phenomenon occurs when the competitively dominant taxa in a community are those that contribute least to the measured ecosystem function. Three lines of evidence from my experiments suggest that the negative selection effect does not occur in late-stage source communities. First, I did not detect any trends towards increasing rates of competitive exclusions in late-stage source communities. Second, these communities had some of the smallest nonlinear richness (i.e., species interaction) terms and extents of inhibition. These lines of evidence signify a low contribution of negative species interactions

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

to the diminished respiration in late-stage pitchers [39]. Further study, however, is needed to determine 1) whether observed successional decreases in competition result from decreasing niche overlap within late-stage pitcher communities and 2) the relative influence of competition versus habitat filtering during different stages of ecosystem development and how these factors, acting historically, contribute to contemporary community structure.

Conclusions

All previous experimental studies measuring the BEF relationship over time do so using communities with finite resources and no immigration. Consequently, the closed nature of these systems may have influenced the resulting community dynamics and ecosystem processes. My study, however, measured individual "snapshots" of communities assembled from a temporal gradient of natural, open source pools. Furthermore, my microcosms were assembled with equal starting concentrations of bacterial strains and resources, which may have prevented communities from becoming resource limited prior to measuring their respirations. Despite these differences, however, decreases in microbial BEF relationships of both static species pools over time and dynamic species pools at a single time point suggest that similar ecological processes may govern these patterns in microbial communities.

In leaves of the pitcher plant *Darlingtonia californica,* bacterial degradation of organic matter is a process critical for the uptake of prey-derived nitrogen and phosphorous in the nutrient-poor habitats to which these plants are adapted. Using bacterial strains isolated from pitcher leaves at regular intervals over a one-year period, I determined the magnitude of the BEF relationship to peak in mid-successional communities. This positive richness effect on respiration was driven primarily by strains' relatively equivalent contributions to ecosystem function. At the same time, respiration was constrained by diffuse competition

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

among strains in polyculture. This study represents an initial attempt to integrate biodiversity-ecosystem function effects over successional time and concludes that the functional consequences of diversity loss on a host or ecosystem may vary along a successional gradient. Future studies on biodiversity-ecosystem function relationships are encouraged to adopt a dynamic species pool framework to improve the generalizability of their results.

DATA, CODE, AND MATERIALS

The datasets supporting this article have been uploaded as a part of the supplementary material.

AUTHOR CONTRIBUTIONS

D.W.A. conceived of the study, collected all data, performed the analyses, and drafted the manuscript.

ACKNOWLEDGEMENTS

I thank H. Miller, A. Petrosky & R. Leon for assistance with data collection and E. Simms for providing key equipment. W. Sousa, M. Power, M. Firestone, and J. Lefcheck provided helpful comments. Funding was provided by NSF DEB-1406524 & NSF GRFP.

REFERENCES

1. Odum, E. P. 1969 The strategy of ecosystem development. *Science* **164**, 262–270. (doi:10.1126/science.164.3877.262)

2. DeAngelis, D. L. 1992 *Dynamics of Nutrient Cycling and Food Webs*. London ; New York: Chapman & Hall.

3. Loreau, M. 2010 *From Populations to Ecosystems: Theoretical Foundations for a New Ecological Synthesis: Theoretical Foundations for a New Ecological Synthesis*. Princeton, NJ: Princeton University Press.

4. Loreau, M. et al. 2001 Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* **294**, 804–808. (doi:10.1126/science.1064088)

5. Hooper, D. U. et al. 2005 Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* **75**, 3–35. (doi:10.1890/04-0922)

6. Cardinale, B. J., Srivastava, D. S., Emmett Duffy, J., Wright, J. P., Downing, A. L., Sankaran, M. & Jouseau, C. 2006 Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* **443**, 989–992. (doi:10.1038/nature05202)

7. Loreau, M. & Hector, A. 2001 Partitioning selection and complementarity in biodiversity experiments. *Nature* **412**, 72–76. (doi:10.1038/35083573)

8. Turnbull, L. A., Levine, J. M., Loreau, M. & Hector, A. 2013 Coexistence, niches and biodiversity effects on ecosystem functioning. *Ecol. Lett.* **16**, 116–127. (doi:10.1111/ele.12056)

9. Connell, J. H. & Sousa, W. P. 1983 On the evidence needed to judge ecological stability or persistence. *Am. Nat.* **121**, 789–824. (doi:10.1086/284105)

10. Scheffer, M. 2009 *Critical Transitions in Nature and Society*. Princeton, NJ: Princeton University Press.

11. Connell, J. H. & Slatyer, R. O. 1977 Mechanisms of succession in natural communities and their role in community stability and organization. *Am. Nat.* **111**, 1119–1144. (doi:10.1086/283241)

12. Tilman, D. 1988 *Plant Strategies and the Dynamics and Structure of Plant Communities*. Princeton, NJ: Princeton University Press.

13. Grime, J. P. 1979 *Plant strategies and vegetation processes*. 1st edn. Chichester: Wiley.

14. Kinzig, A. P. & Pacala, S. W. 2001 Successional biodiversity and ecosystem functioning. In *The functional consequences of biodiversity: empirical progress and theoretical extensions* (eds A. P. Kinzig S. W. Pacala & G. D. Tilman), pp. 175–212. Princeton, NJ: Princeton University Press.

15. Mouquet, N., Moore, J. L. & Loreau, M. 2002 Plant species richness and community productivity: why the mechanism that promotes coexistence matters. *Ecol. Lett.* **5**, 56–65. (doi:10.1046/j.1461-0248.2002.00281.x)

16. Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T. & Lehman, C. 2001 Diversity and productivity in a long-term grassland experiment. *Science* **294**, 843–845. (doi:10.1126/science.1060391)

17. Fox, J. W. 2004 Effects of algal and herbivore diversity on the partitioning of biomass within and among trophic levels. *Ecology* **85**, 549–559. (doi:10.1890/03-0095)

18. Bell, T., Newman, J. A., Silverman, B. W., Turner, S. L. & Lilley, A. K. 2005 The contribution of species richness and composition to bacterial services. *Nature* **436**, 1157–1160. (doi:10.1038/nature03891)

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

19. Ruijven, J. van & Berendse, F. 2005 Diversity–productivity relationships: Initial effects, long-term patterns, and underlying mechanisms. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 695–700. (doi:10.1073/pnas.0407524102)

20. Spehn, E. M. et al. 2005 Ecosystem effects of biodiversity manipulations in European grasslands. *Ecol. Monogr.* **75**, 37–63. (doi:10.1890/03-4101)

21. Cardinale, B. J., Wright, J. P., Cadotte, M. W., Carroll, I. T., Hector, A., Srivastava, D. S., Loreau, M. & Weis, J. J. 2007 Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proc. Natl. Acad. Sci.* **104**, 18123–18128. (doi:10.1073/pnas.0709069104)

22. Weis, J. J., Cardinale, B. J., Forshay, K. J. & Ives, A. R. 2007 Effects of species diversity on community biomass production change over the course of succession. *Ecology* **88**, 929–939. (doi:10.1890/06-0943)

23. Doherty, J. M., Callaway, J. C. & Zedler, J. B. 2011 Diversity–function relationships changed in a long-term restoration experiment. *Ecol. Appl.* **21**, 2143–2155. (doi:10.1890/10-1534.1)

24. Gravel, D., Bell, T., Barbera, C., Bouvier, T., Pommier, T., Venail, P. & Mouquet, N. 2011 Experimental niche evolution alters the strength of the diversity-productivity relationship. *Nature* **469**, 89–92. (doi:10.1038/nature09592)

25. Reich, P. B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S. E., Flynn, D. F. B. & Eisenhauer, N. 2012 Impacts of biodiversity loss escalate through time as redundancy fades. *Science* **336**, 589–592. (doi:10.1126/science.1217909)

26. Lasky, J. R., Uriarte, M., Boukili, V. K., Erickson, D. L., John Kress, W. & Chazdon, R. L. 2014 The relationship between tree biodiversity and biomass dynamics changes with tropical forest succession. *Ecol. Lett.* **17**, 1158–1167. (doi:10.1111/ele.12322)

27. Jiang, L. 2007 Negative selection effects suppress relationships between bacterial diversity and ecosystem functioning. *Ecology* **88**, 1075–1085. (doi:10.1890/06-1556)

28. Salles, J. F., Poly, F., Schmid, B. & Le Roux, X. 2009 Community niche predicts the functioning of denitrifying bacterial assemblages. *Ecology* **90**, 3324–3332. (doi:10.1890/09-0188.1)

29. Langenheder, S., Bulling, M. T., Sloan, M. & Prosser 2010 Bacterial biodiversity-ecosystem functioning relations are modified by environmental complexity. *PLOS ONE* **5**, e10834. (doi:10.1371/journal.pone.0010834)

30. Venail, P. A. & Vives, M. J. 2013 Phylogenetic distance and species richness interactively affect the productivity of bacterial communities. *Ecology* **94**, 2529–2536. (doi:10.1890/12-2002.1)

31. Redford, A. J. & Fierer, N. 2009 Bacterial succession on the leaf surface: A novel system for studying successional dynamics. *Microb. Ecol.* **58**, 189–198. (doi:10.1007/s00248-009-9495-y)

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

476 32. Armitage, D. W. 2016 Microbes in time: incorporating bacteria into ecosystem
477 development theory. Ph.D dissertation, University of California Berkeley.

478 33. Lloyd, F. E. 1942 The Carnivorous Plants. In *Chronica Botanica*, New York, NY:
479 Ronald Press.

480 34. Juniper, B. B. E., Robins, R. J. & Joel, D. M. 1989 *The Carnivorous Plants*. London,
481 UK; San Diego, CA: Academic Press.

482 35. Hepburn, J. S., Jones, F. M. & John, E. Q. 1927 The biochemistry of the American
483 pitcher plants: biochemical studies of the North American Sarraceniaceae. *Trans.*
484 *Wagner Free Inst. Sci. Phila.* **1927**, 1–95.

485 36. Lindquist, J. A. 1975 Bacteriological and ecological observations on the northern pitcher
486 plant, *Sarracenia purpurea* L.

487 37. Butler, J. L., Gotelli, N. J. & Ellison, A. M. 2008 Linking the brown and green: nutrient
488 transformation and fate in the *Sarracenia* microecosystem. *Ecology* **89**, 898–904.
489 (doi:10.1890/07-1314.1)

490 38. Petrosky, A. 2015 Local adaptation of bacteria and bacteriophage in California pitcher
491 plants (*Darlingtonia californica*). Honor's Thesis, University of California Berkeley.

492 39. Bell, T., Lilley, A. K., Hector, A., Schmid, B., King, L. & Newman, J. A. 2009 A linear
493 model method for biodiversity–ecosystem functioning experiments. *Am. Nat.* **174**, 836–
494 849. (doi:10.1086/647931)

495 40. Foster, K. R. & Bell, T. 2012 Competition, not cooperation, dominates interactions
496 among culturable microbial species. *Curr. Biol.* **22**, 1845–1850.
497 (doi:10.1016/j.cub.2012.08.005)

498 41. Keddy, P. A. 2012 *Competition*. New York: Springer Science & Business Media.

499 42. Rivett, D. W., Scheuerl, T., Culbert, C. T., Mombrikotb, S. B., Johnstone, E.,
500 Barraclough, T. G. & Bell, T. 2016 Resource-dependent attenuation of species
501 interactions during bacterial succession. *ISME J.* (doi:10.1038/ismej.2016.11)

502 43. Huston, M. A. 1997 Hidden treatments in ecological experiments: re-evaluating the
503 ecosystem function of biodiversity. *Oecologia* **110**, 449–460.
504 (doi:10.1007/s004420050180)

505 44. Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S. & Potts, J. M. 2003
506 A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate
507 amendments so as to determine the physiological profiles of soil microbial communities
508 by using whole soil. *Appl. Environ. Microbiol.* **69**, 3593–3599.
509 (doi:10.1128/AEM.69.6.3593-3599.2003)

510 45. R Development Core Team 2015 *R: A language and environment for statistical*
511 *computing*. Vienna, Austria: R Foundation for Statistical Computing.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

46. Fish, D. & Hall, D. W. 1978 Succession and stratification of aquatic insects inhabiting the leaves of the insectivorous pitcher plant, *Sarracenia purpurea*. *Am. Midl. Nat.* **99**, 172–183. (doi:10.2307/2424941)

47. Nemergut, D. R. et al. 2015 Decreases in average bacterial community rRNA operon copy number during succession. *ISME J.* (doi:10.1038/ismej.2015.191)

48. Fiegna, F., Moreno-Letelier, A., Bell, T. & Barraclough, T. G. 2015 Evolution of species interactions determines microbial community productivity in new environments. *ISME J.* **9**, 1235–1245. (doi:10.1038/ismej.2014.215)

49. Lawrence, D., Fiegna, F., Behrends, V., Bundy, J. G., Phillimore, A. B., Bell, T. & Barraclough, T. G. 2012 Species interactions alter evolutionary responses to a novel environment. *PLoS Biol* **10**, e1001330. (doi:10.1371/journal.pbio.1001330)

50. Harmon, L. J., Matthews, B., Des Roches, S., Chase, J. M., Shurin, J. B. & Schluter, D. 2009 Evolutionary diversification in stickleback affects ecosystem functioning. *Nature* **458**, 1167–1170. (doi:10.1038/nature07974)

51. Zuppinger-Dingley, D., Schmid, B., Petermann, J. S., Yadav, V., De Deyn, G. B. & Flynn, D. F. B. 2014 Selection for niche differentiation in plant communities increases biodiversity effects. *Nature* **515**, 108–111. (doi:10.1038/nature13869)

52. Ellison, A. M., Gotelli, N. J., Brewer, J. S., Cochran-Stafira, D. L., Kneitel, J. M., Miller, T. E., Worley, A. C. & Zamora, R. 2003 The evolutionary ecology of carnivorous plants. *Adv. Ecol. Res.* **33**, 1–74. (doi:10.1016/S0065-2504(03)33009-0)

53. Wacker, L., Baudois, O., Eichenberger-Glinz, S. & Schmid, B. 2009 Diversity effects in early- and mid-successional species pools along a nitrogen gradient. *Ecology* **90**, 637–648. (doi:10.1890/07-1946.1)

54. Petermann, J. S., Fergus, A. J. F., Roscher, C., Turnbull, L. A., Weigelt, A. & Schmid, B. 2010 Biology, chance, or history? The predictable reassembly of temperate grassland communities. *Ecology* **91**, 408–421. (doi:10.1890/08-2304.1)

FIGURE LEGENDS

Figure 1. Species pools for BEF experiments are typically chosen either by sampling a community at a single point in time (a) or from a group of taxa which may not co-occur at a particular time point (b). Far fewer studies have taken the approach of measuring BEF relationships over a temporally dynamic species pool (c).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

545 **Figure 2**. Relationships between strain richness and community respiration for synthetic
546 bacterial communities assembled from pitchers of different ages. Black lines denote
547 significant linear richness fits for individual communities within each age group ($p < 0.05$).
548 Mean values for the response variables are presented for clarity. Bars denote standard error
549 measurements.
550
551 **Figure 3.** (a) Linear richness (β_{LR}) regression coefficients as a function of source community
552 age. Bars denote 95% confidence intervals and shared letters between ages signify an overlap
553 between the two estimates. Asterisks (*) denote coefficients found to be significantly greater
554 than zero (F-test, $p < 0.05$). (b) Log mean square estimates for the species impact (β_i) and
555 nonlinear richness (β_{NLR}) parameters. These values represent the relative contributions of
556 species-specific effects and species interactions, respectively, on respiration rates. None of
557 these coefficients were significantly greater than zero.
558
559 **Figure 4.** Relative inhibition of bacterial respiration in polycultures measured as the
560 difference between additive predicted and observed rates. Values of zero indicate that the
561 sum of community members' respirations in monoculture equaled the community's
562 performance in polyculture. Values greater than zero indicate that observed rates were less
563 than predicted rates and provide evidence for interspecific competitive or antagonistic
564 inhibition. The Y-axis has been reversed to more clearly illustrate this inhibition. Letters
565 shared by points within a richness group indicate that their means (white points) do not
566 significantly differ from one another (Tukey's range test, $p < 0.05$). Shading denotes richness
567 treatments.
568
569

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

570 **TABLES**

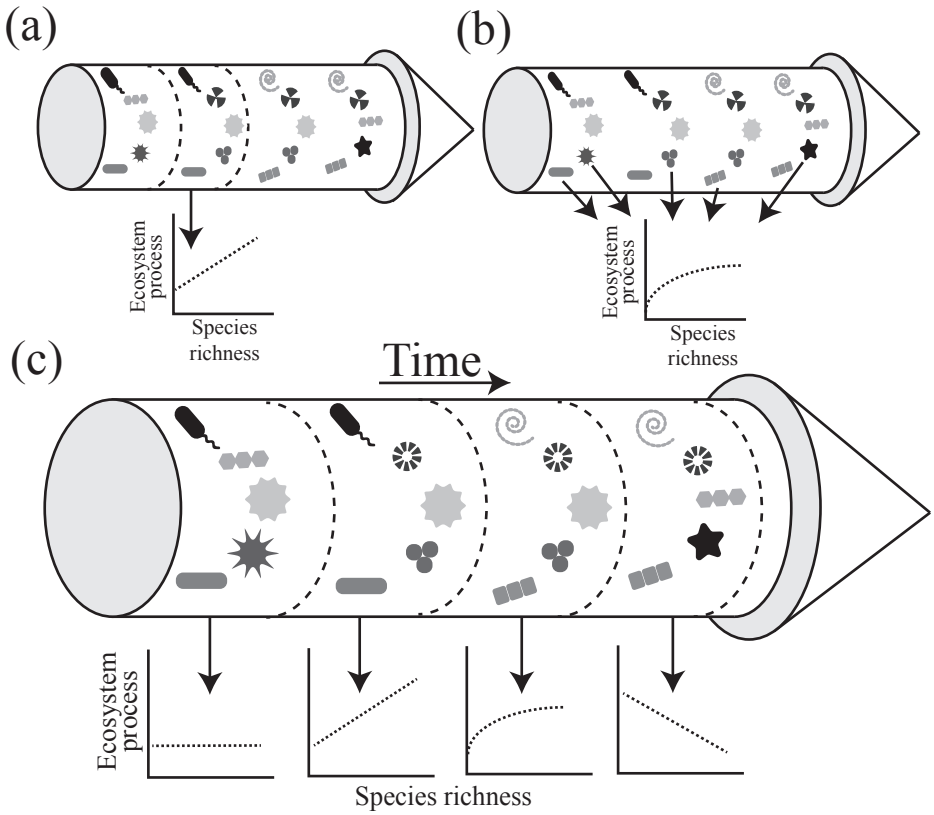
571 **Table 1**. ANOVA and ANCOVA results for total respiration and respiration differences (i.e.
572 interspecific inhibition). Respiration rates were log-transformed to satisfy homoscedasticity.
573 Richness was treated as a continuous variables and age as a categorical variable with
574 contrasts summing to zero. Marginal (type 3) sums-of-squares (SS) are presented.

Response	Covariate	DF	SS	*F*	*p*	R^2
Log respiration rate	Intercept	1	17.35	317.35	< 0.001	0.17
	Source community age	8	12.89	12.89	< 0.001	
	Species richness	1	47.03	47.03	< 0.001	
	Interaction term	8	14.52	3.311	< 0.001	
	Residuals	1926	1056			
Response	**Covariate**	**DF**	**SS**	***F***	***p***	**R^2**
Expected - observed respiration	Intercept	1	689	154	< 0.001	0.88
	Source community age	8	1050	29.3	< 0.001	
	Species richness	1	7481	1675.0	< 0.001	
	Interaction term	8	1133	31.7	< 0.001	
	Residuals	270	1206			

575
576
577
578
579
580
581
582
583
584
585
586
587

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

588 Figure 1



589

590

591

592

593

594

595

596

597

598

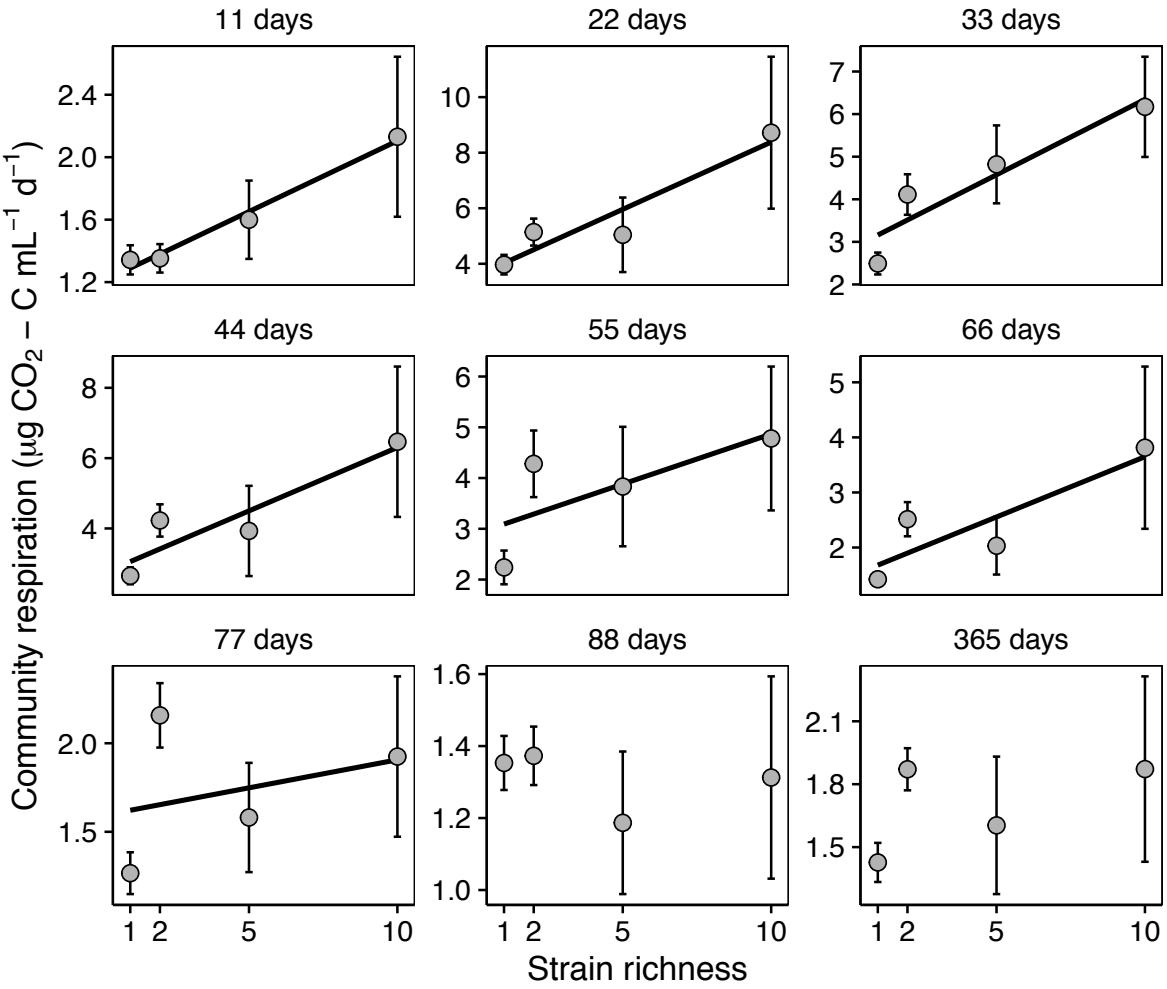
599

600

601

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

602 Figure 2



603

604

605

606

607

608

609

610

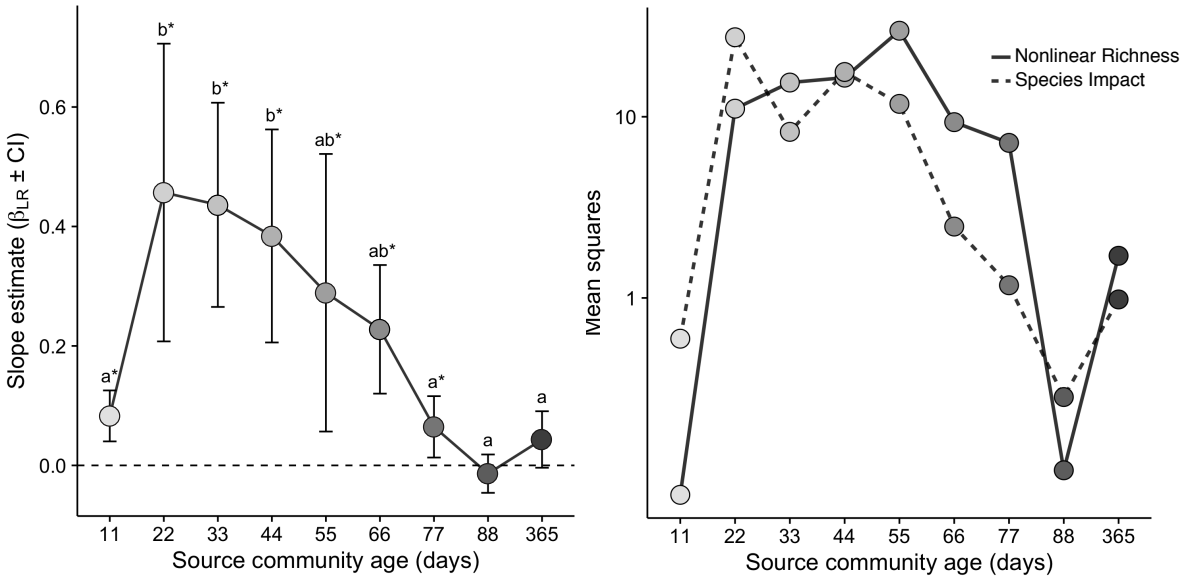
611

612

613

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

614 Figure 3



615

616

617

618

619

620

621

622

623

624

625

626

627

628

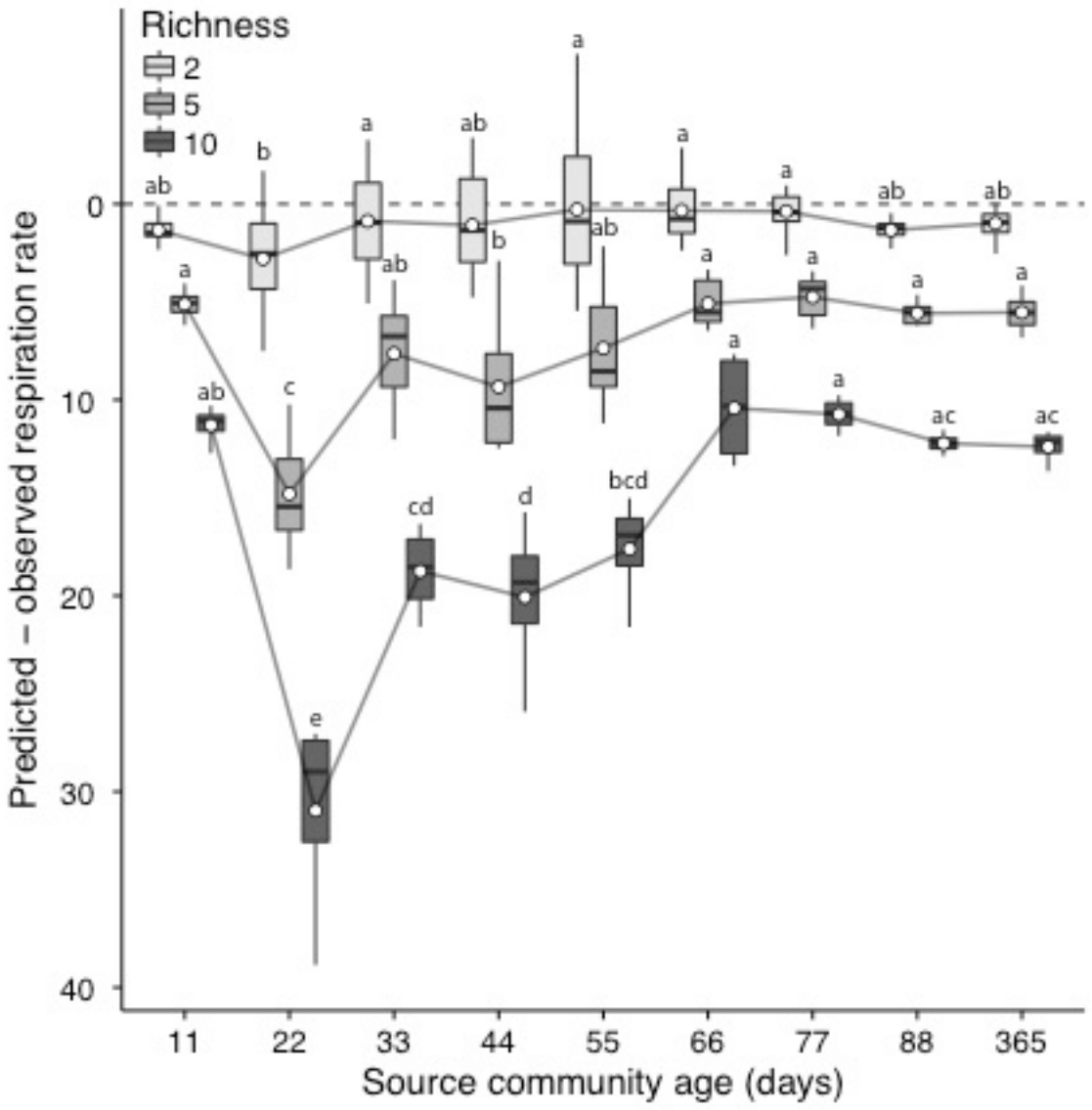
629

630

631

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016

632 Figure 4



633

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.1897v2 | CC BY 4.0 Open Access | rec: 23 Aug 2016, publ: 23 Aug 2016