

1 Time-variant species pools shape competitive dynamics and diversity-ecosystem
2 function relationships in pitcher plant bacterial communities

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26 Abstract

27 Biodiversity-ecosystem function (BEF) experiments often employ common garden designs,
 28 drawing samples from a local biota. However, the communities from which taxa are sampled
 29 may not be at equilibrium. I assembled pools of aquatic bacterial strains isolated at different
 30 time points from leaves on the pitcher plant *Darlingtonia californica* to evaluate the role of a
 31 dynamic, host-associated microbiota on the BEF relationship. I constructed experimental
 32 communities using bacteria from each time point and measured their respiration rates.
 33 Communities assembled from mid-successional species pools showed the strongest positive
 34 relationships between community richness and respiration rates, driven primarily by linear
 35 additivity among isolates. Diffuse competition was common among all communities but
 36 greatest within mid-successional isolates. These results demonstrate the dependence of the
 37 BEF relationship on the temporal dynamics of the local species pool, implying that
 38 ecosystems may respond differently to the addition or removal of taxa at different points in
 39 time during succession.

40

41 INTRODUCTION

42 The rates at which ecosystems recycle energy and nutrients are predicted to be set in large
 43 part by the actions of their constituent organisms (Odum 1969; DeAngelis 1992; Loreau
 44 2010). Over the past two decades, this conceptual unification of community and ecosystem
 45 scales has been empirically evaluated using the biodiversity - ecosystem function (BEF)
 46 framework (Loreau *et al.* 2001; Hooper *et al.* 2005; Cardinale *et al.* 2006). This research
 47 commonly reports a positive covariance between species richness and community biomass
 48 production and is hypothesized to be jointly driven by community members' differential
 49 contributions to ecosystem properties (selection effects) and their degree of niche overlap
 50 (complementarity effects) (Loreau & Hector 2001). Because bacteria play fundamental roles

in global nutrient cycles (Falkowski *et al.* 2008), it is important to understand how the dynamical nature of their communities influences rates of elemental flux. In addition, it is becoming increasingly clear that the taxonomic and functional composition of host-associated microbiota change over time, with implications for the host's fitness (Redford & Fierer 2009; Koenig *et al.* 2011). Thus, a comprehensive theory linking microbial diversity to ecosystem function must explicitly consider the effects of species turnover through time (Kinzig & Pacala 2001; Mouquet *et al.* 2002).

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 experimentally 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 (Tilman *et al.* 2001; Fox 2004; Bell *et al.* 2005; Ruijven & Berendse 2005; Spehn *et al.* 2005; Cardinale *et al.* 2007; Weis *et al.* 2007; Doherty *et al.* 2011; Gravel *et al.* 2011; Reich *et al.* 2012;). While these experiments have contributed fundamental insights into the temporal dynamics of diversity-ecosystem function 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 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 most biodiversity-ecosystem function experiments have been conducted using plant and algal communities, the framework has also been successfully extended to other taxonomic groups (e.g., van der Heijden *et al.* 1998; Lefcheck & Duffy 2015). In particular, bacterial communities have been the subject of numerous BEF studies, owing to their importance in regulating ecosystem processes (Bell *et al.* 2005; Jiang 2007; Salles *et al.* 2009; Langenheder *et al.* 2010; Gravel *et al.* 2011; Venail & Vives 2013). But no study to date has considered the effects of a dynamic, successional bacterial community on ecosystem functioning. Furthermore, the BEF framework has yet to be extended to host-associated bacterial communities, which often exhibit marked turnover through time (e.g., Redford & Fierer 2009; Koenig *et al.* 2011) and can positively or negatively influence host fitness (e.g., Lugtenberg & Kamilova 2009; Thomas *et al.* 2010).

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, fluid-filled leaf in which trapped insects are drowned (Lloyd 1942; Juniper *et al.* 1989). Digestion is facilitated both by enzymes produced by the plant (Hepburn *et al.* 1927; Gallie & Chang 1997) and by the bacteria residing in the fluid (Hepburn *et al.* 1927; Lindquist 1975; Butler *et al.* 2008). These bacterial communities are dynamic and change predictably over time such that they are more similar between two leaves at the same time point than within a single leaf at two different points in time (Koopman *et al.* 2010).

The pitcher plant *Darlingtonia californica* (Torr.) is hypothesized to rely heavily on bacteria for prey digestion (Hepburn *et al.* 1927). The pitcher leaves of this species are produced at regular intervals throughout the June-October growing season and are sterile

prior to opening. Once the leaves fully develop, they quickly begin trapping insects, and bacterial biomass skyrockets to over 10^9 cells mL^{-1} (Armitage, unpublished data). After approximately two months, a leaf slowly ceases to capture prey yet remains photosynthetically active for a second growing season. Bacterial diversity in *Darlingtonia* pitchers also changes over time, as has been documented by both culture-independent molecular approaches as well as among bacterial cultures isolated from different aged leaves (Armitage, unpublished data). These temporal isolates provide a unique opportunity to experimentally test the 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. I anticipated results mirroring those of Lasky *et al.* (2014) and Weis *et al.* (2007), wherein the positive effect of species richness on ecosystem function decreased during succession. Second, I used these data to estimate the relative influences of individual species and their interspecific interactions (such as competition) on the BEF relationship (Bell *et al.* 2009). 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 (Foster & Bell 2012). 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 (Foster & Bell 2012). 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 (Connell & Slatyer 1977). Alternatively, the bacterial taxa dominating late-stage pitchers may be specialists on recalcitrant carbon resources and therefore may not contribute significantly to ecosystem function, compared to early, fast-growing colonists. 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.

MATERIAL AND METHODS

Sample collection & strain isolation

I collected samples during 2014 in a single large patch of *Darlingtonia californica* pitcher plants growing in the Butterfly Valley Botanical Area, Plumas National Forest (Plumas Co, CA). *Darlingtonia* plants begin producing leaves in mid-June, which remain closed and sterile until they reach their maximum height. At the start of the growing season in 2014, I identified and tagged 5 emerging pitcher leaves of equivalent developmental stage. The exact date of each pitcher's opening was recorded, allowing me to return to tagged leaves throughout their two-year lifespan and know the precise age of their associated communities. I visited this cohort of leaves every 11 days to collect samples of pitcher fluid. To remove pitcher fluid without damaging the leaf, I used an insect aspirator connected to a flexible tube to siphon pitcher fluid into a sterile centrifuge tube. I performed this on all 5 pitchers, making sure to sterilize the tubing and rubber stopper with 70% ethanol and a 4% bleach-tween solution between samples. The centrifuge tubes were placed on ice and immediately returned to the lab. I replaced the fluid collected from each pitcher with an equal volume of sterile

phosphate buffer (PBS; pH 6) to minimize damage to the leaf tissue and its associated aquatic food web.

I spread dilutions of all five leaves onto R2A agar (pH 6) and incubated them at 25° C. After 48 hours, I isolated all visually distinct colony phenotypes onto their own plates based on color and colony morphology. I verified the genetic basis for these isolates' colony morphologies by serially re-plating them three times and observing no phenotypic changes. I selected bacterial strains to be used in the experiment based on growth rates and distinct colony morphologies. All chosen strains attained a minimum colony diameter of 5 mm on R2A plates and an optical density at 600 nm (OD₆₀₀) of at least 0.5 in R2A broth after 48 hours of shaken culture.

From this remaining pool of bacterial isolates, I selected the 10 most abundant strains to represent the pool of isolates for a given pitcher age. I repeated this process using the same five pitcher leaves every 11 days until day 88. I collected an additional sample of 5 one-year-old pitcher leaves from the 2013 season. Pooling pitchers of a given age was necessary, as there were never 10 distinct cultivable phenotypes fitting my selection criteria within a single leaf. Despite this caveat, strain overlap among pitchers appeared high based on colony morphology, and both plating and rRNA amplicon surveys of pitchers revealed their communities to strongly cluster by age (Koopman *et al.* 2010, Armitage, unpublished data). In a study from the same population of *Darlingtonia*, 147 bacterial isolates were isolated from 44-day and 365-day pitchers using the same methods as this study. These strains were assigned a taxonomy based on their 16s ribosomal rRNA genes. The genera of nearly every isolate matched with the 25 most abundant taxa recovered from culture-independent 16s amplicon sequencing of similar-aged pitchers (Armitage, unpublished data). To estimate a

leaf's rate of prey capture, I collected prey material from 10 pitcher leaves of identical age and divided its dry mass by the number of days it had been open. Supplemental 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. (2009). My experiment consisted of 4 partitions (P), each containing 4 species 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 design ensures that a single species is not overrepresented within any richness level. 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. This enables the user to estimate species' contributions to emergent ecosystem properties (e.g. carbon mineralization rates). Furthermore, it relaxes the requirement for a full-factorial experimental design, which becomes intractable as the number of species increases. In total, I assembled 216 communities per time point, resulting in a total of 1944 cultures spanning 9 source community ages and 4 levels of richness.

The bacterial strains used to seed the experimental communities were grown in shaken R2A broth to mid-log phase and diluted using PBS to an OD_{600} of 0.25. Aliquots of these isolates were then added into microcentrifuge tubes and centrifuged at $10,000 \times G$ for 5 minutes at $4^\circ C$. Next, I removed the supernatant broth from the tubes and washed the pellet of R2A medium by adding 1 mL sterile PBS, re-suspending the pellet, and centrifuging it again. This step was repeated twice and the pellets were left to starve for 2 hours at room

temperature to consume any residual medium. These pellets were then agitated and seeded into 1.2 mL 96-well plates containing 700 μL of sterile growth medium. This medium contained an M9 salt solution (NH_4Cl 1 g L^{-1} , Na_2HPO_4 6 g L^{-1} , KH_2PO_4 3 g L^{-1} , NaCl 0.5 g L^{-1} , pH 6.0) into which I added 3 g L^{-1} of powder from freeze-dried crickets that had been ground and autoclaved. Each strain was then introduced into its community at the volume required to keep the total number of cells across richness treatments equal (100 μL total inoculum). Once assembled, plates were sealed using a sterile, perforated rubber gasket. Over this gasket I placed an inverted 96-well plate containing cresol red dye and NaHCO_3 set in 1% purified agar. CO_2 respired by the microbial communities passes through the perforated gasket where it reacts with the agar following the equation $\text{CO}_2 + \text{H}_2\text{O} + \text{HCO}_3^- \rightarrow 2 \text{CO}_3^- + 3 \text{H}^+$. This redox reaction induces a colorimetric change in the dye that can be read on a 96-well spectrophotometer (see Campbell *et al.* 2003).

All experimental communities from a source pitcher age were run simultaneously on at 25° C for three days. I estimated percentage of CO_2 -C in each agar well by measuring its absorbance at 570 nm and comparing these values to a calibration curve. Next, I estimated the rate of carbon respired by each community using the ideal gas formula

$$\mu\text{g CO}_2 - \text{C mL}^{-1} \text{ d}^{-1} = \left\{ \frac{\frac{\% \text{CO}_2}{100} \cdot H \cdot \frac{44}{22.4} \cdot \frac{12}{44} \cdot \frac{273}{273 + T}}{V} \right\} \cdot \frac{1}{t} \quad (1)$$

where H is the headspace volume of the culture well (400 μL), T is the temperature (25° C), V is the volume of medium (800 μL), and t is the incubation time (3 days). After three days, I removed the colonies and plated the 10-species communities onto agar in order to assess whether extinctions had taken place. This procedure was repeated every 11 days using different pools of isolates collected from the same pitchers.

Statistical analyses

To assess how drivers of the BEF relationship differed among time points, I fit a linear model to community respiration rates (Bell *et al.* 2009). This model took the form

$$y = \beta_0 + \beta_{LR}x_{LR} + \beta_{NLR}x_{NLR} + \left(\sum_i^S \beta_i x_i \right) + \beta_Q x_Q + \beta_M x_M + \varepsilon \quad (2)$$

where y is a community or ecosystem process (e.g., respiration rate), β_{LR} is the effect of species richness measured on a continuous scale (linear richness, x_{LR}), β_{NLR} is the effect of species richness measured on a categorical scale (non-linear richness, x_{NLR}), β_i is the impact of an individual species presence in the productivity of its community, β_Q is the effect of the particular species pool used in each $P \times R$ treatment, and β_M is the effect of a particular species composition within each species pool, β_0 is the intercept, and ε is the error term.

By estimating the linear richness term prior to the nonlinear richness and species' impact terms, the latter two terms become orthogonal. The species impact (β_i) terms sum to zero and reflect the relative influence an individual strain exerts on the community's respiration. The nonlinear richness term (β_{NLR}) can be interpreted as the magnitude of deviations from linear species richness effects. Nonzero values of β_{NLR} reflect the influence of facilitative and competitive interactions on ecosystem processes when all pairwise interactions among species cannot be statistically evaluated. I used least squares to estimate the model coefficients and an F-test to determine the statistical significance of each variable. Because species impact models consisted of 10 parameters, the overall significance of the parameterized β_{NLR} and β_i models were assessed using a likelihood ratio test against intercept-only null models. Model terms were entered in the order in which they appear in equation 2: nonlinear richness (β_{NLR}) and species impacts (β_i) were estimated from the residuals of the model containing the linear richness (β_{LR}) term.

246

247 I estimated the effects of the source pitchers' ages and prey capture rates, as well as
248 experimental communities' richness on rates of CO₂ respiration using linear regression.
249 Source community age and prey capture rate were perfectly collinear and so their effects
250 could not be simultaneously estimated. To aid in the interpretation of interactions, predictors
251 were centered to their mean values prior to model fitting. I assessed the pairwise differences
252 among community ages using Tukey's range test ($\alpha = 0.05$).

253

254 I estimated the extent to which strains inhibit one another's potential CO₂ production
255 in polyculture by calculating the difference between a community's predicted and observed
256 respiration rates. The predicted values were calculated by summing all community members'
257 average monoculture respiration rates. The difference between a polyculture's predicted and
258 observed respiration values will equal zero if there are no inhibitory effects between
259 members of the community (i.e., all taxa in a polyculture perform as well they do in
260 monoculture). Alternatively, direct antagonism (e.g., antibiotic production) or resource
261 competition is anticipated to result in respiration rates less than the additive prediction.
262 Similarly, facilitative interactions such as cross feeding are expected to increase polyculture
263 respiration compared to predicted rates (Foster & Bell 2012). I used ANCOVA to test the
264 null hypothesis that the mean differences between predicted and observed respiration rates
265 were equal among community ages, controlling for richness effects. Pairwise differences
266 between centered predictor variables were assessed using Tukey's range test. All models
267 were fit using R v3.1 (R Development Core Team 2015).

268

269 *Community metabolic fingerprinting*

I measured the carbon metabolic profiles of each 10-species community using the GN2 microplate assay (Biolog, Inc.). I inoculated each dilute, starved, 10-strain mixture onto the 96-well GN2 plate, which consisted of 95 unique carbon compounds and a blank. The inoculum contained dye that turns violet when reduced by NADPH. The optical absorbance at 570 nm of these wells is proportional to the productivity of the community on the particular substrate. The pattern of metabolite use provides a unique metabolic “fingerprint” that can serve as a basis for the comparison of different communities. I performed this assay on each 10-species community in triplicate. After three days of incubation at 25° C I visually scored each well. If a violet color had developed in the same well across the three replicates, I scored that substrate as an electron donor for at least one strain in the community. With these data, I constructed a logical matrix from which I calculated the pairwise Jaccard distances between each community’s carbon metabolic profiles. Next, I ordinated these distances using Principal Coordinates Analysis (PCoA) and visually assessed the results on a plot of the first two principal coordinates.

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 and letting them dry for three hours. Onto these lawns I spotted 2 µL log-phase broth culture of each co-occurring isolate using a flame sterilized 48-pin replicator. 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 using a dissecting microscope. I considered the spotted strain to be inhibitory to the focal strain if unambiguous zones of clearing surrounded at least 6 replicate spots.

295

296 RESULTS

297 After 3 days, I found an average of 6.9 (se = 0.18, range = 5-9) bacterial strains remaining in
 298 each 10-species community, and there were no significant differences in the proportions of
 299 surviving strains among source community ages ($F_{8,27} = 2.3$, $p = 0.06$). Thus, although the
 300 strains' relative abundances changed throughout the incubation period, no single, dominant
 301 strain was able to exclude the majority of others. I detected significant differences between
 302 the mean respiration rates of bacterial communities isolated from pitcher leaves of different
 303 ages (table 1, fig. 2). Post-hoc analysis revealed respiration rates to be greatest among
 304 bacterial communities isolated from pitcher leaves between 22 and 55 days old (fig. 2). This
 305 pattern was consistent under all four richness treatments, although there was a general
 306 tendency for variance in respiration rates among treatments to increase when more strains
 307 were present (fig. 2). Bacterial richness had a significantly positive effect on overall
 308 respiration rates, independent of age ($\beta_R = 0.05 \pm 0.007$; table 1), although there was a
 309 significant interaction between richness and source community age (table 1). Likewise, the
 310 mean prey capture rate of pitchers within an age cohort was significantly positively
 311 associated with overall rates of carbon respiration ($\beta_{capture} = 0.06 \pm 0.012$; table 1).

312

313 The effect of linear richness (β_{LR}) on respiration rates was significantly positive for all
 314 source community ages except those from days 88 and 365 (fig. 3). This positive effect of
 315 richness on respiration was greatest for isolates from pitcher leaves between 22 and 66 days
 316 old, and tended to increase from days 11 to 22 and then slowly decrease towards zero
 317 throughout the rest of the pitchers' lifespan (fig. 4A). For each bacterial isolate pool, I
 318 detected both significant nonlinear richness effects (β_{NLR}) and individual species (β_i) effects

(table S1), and the relative influence of nonlinear richness effects was greater than overall species effects for the majority of time points (fig. 4B).

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. 5). The magnitude of this inhibitory effect increased with species richness (fig. 5, table 1). I only detected 12 positive 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. 6A) or in their multivariate Jaccard similarities (fig. 6B).

DISCUSSION

Dynamic species pools impact BEF relationships

I encountered a unimodal association between the age of the bacterial source community and 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 rates during this period coincided with the greatest rates of prey capture by the pitcher leaf. It is possible that the relatively low respiration rates of late-stage source communities reflect an adaptive strategy for living in nutrient-replete environment. This is supported by the observation of lower average ribosomal RNA copy numbers — a trait correlated with growth rate— as succession proceeds (Nemergut *et al.*

2015). However, information on all strains' relative performances across different nutrient concentrations would be required to experimentally verify this hypothesis.

The effects of a community's richness on rates of respiration were generally positive, but tended to vary over time such that the slope estimates were unimodal, peaking in pitcher leaves of intermediate age. These positive BEF relationships appeared to be driven by the linear, additive contributions of taxa, as evidenced by the positive linear richness terms and weak species impact terms. This interpretation is supported by the rarity of both competitive exclusions and direct antagonistic interactions between taxa in all species pools. However, competitive inhibition of a community's potential maximum respiration rate was common in all 5- and 10- species polycultures and peaked in communities assembled from intermediate-aged pitcher leaves — an observation supported by significant nonlinear richness terms in many of the communities. This general pattern of diffuse competition in polycultures may not be typical of bacteria within pitcher plants due to biases in my isolation procedure (Foster & Bell 2012). By using a single medium to isolate bacteria, it is likely that the strains I sampled were more phenotypically similar to one another than to a random sample of all bacteria in a pitcher leaf. Thus, the strains used in this study should be considered members sampled from a guild of aerobic, heterotrophic bacteria and are expected to compete with one another for resources. A useful follow-up to this experiment would investigate the effects of increasing the phenotypic diversity of the species pool by adding strains obtained using a broader range of media.

Competition among isolates is predicted to decrease in bacterial communities over time due to divergent evolution (Lawrence *et al.* 2012). The relatively low levels of competitive inhibition among strains from late-stage pitcher leaves may represent indirect

evidence of divergence. This scenario is plausible, given the rapid generation times and population sizes of the isolates. A recent study by Fiegna *et al.* (Fiegna *et al.* 2015) showed that the experimental evolution of bacterial isolates over 5 weeks can alter the BEF relationship via a relaxation of competition. Although such an effect is possible in natural systems, its demonstration would require tracking individual bacterial lineages over time and regularly assaying their competitive interactions. Miller and Kneitel attempted this by measuring the degree of competitive inhibition of four bacterial colony morphotypes isolated from the same pitchers 7 days and 42 days after opening. The authors found that the competitive abilities (relative to a common bacterial competitor) of two of the four strains decreased with pitcher age while two did not appear to change (Ellison *et al.* 2003). These results match my observation of increased competitive inhibition of potential respiration on a similar timescale (11-day and 44-day leaves).

To date, only one other study has directly estimated the impacts of natural successional dynamics in the context of biodiversity and ecosystem functioning (Lasky *et al.* 2014). Using 15 years of observational data from regenerating tropical forest plots, the authors documented a decreasing effect of species richness on rates of aboveground biomass production in mid- and late-successional tropical forest plots (Lasky *et al.* 2014). These results matched both theoretical predictions (Kinzig & Pacala 2001) and experimental studies in which diversity effects were tracked over time within individual microcosms (Bell *et al.* 2005; Weis *et al.* 2007). My results conform to those of other BEF time-series experiments, despite marked differences in design. In concert, our findings challenge the common observation that the effects of richness on productivity become more positive over time (Cardinale *et al.* 2007), though further investigation is necessary to deduce the mechanisms for these contrasting outcomes.

393

394 *Potential drivers of BEF relationships*

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

405

406 Shifts towards non-significant or negative BEF relationships can be caused by
407 functional redundancy, wherein the majority of taxa within a community are functionally
408 equivalent and the loss of any member will not result in a decrease in ecosystem functioning
409 (Allison & Martiny 2008; Reich *et al.* 2012). In my communities, low variation in respiration
410 among monocultures from 11-day and >66-day source pools supports increased functional
411 redundancy in these communities. This is because functional redundancy requires redundant
412 species to contribute equally to ecosystem function. A community comprised of functionally
413 redundant taxa should also show a marked difference between predicted and observed
414 respiration rates, since redundant taxa will compete for shared carbon resources. In my
415 experiment, however, communities assembled from 11-day and >66-day leaves had relatively
416 low levels of competitive inhibition. Furthermore, a fair assessment of functional redundancy

would require information on the phenotypic overlap of individual strains (Allison & Martiny 2008).

A second mechanism for generating non-positive BEF relationships is the negative selection effect (Loreau & Hector 2001; Jiang 2007; Jiang *et al.* 2008). This phenomenon occurs when the competitively dominant taxa in a community are those that contribute least to the measured ecosystem function. Like the positive selection effect in BEF literature (Loreau & Hector 2001), the negative selection effect implies that competitive interactions will drive communities towards monodominance. Two 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 lowest nonlinear richness (species interaction) effects signifying a low contribution of species interactions to respiration rates.

Conclusions

In leaves of the pitcher plant *Darlingtonia californica*, bacterial carbon mineralization is a process critical for the plant's acquisition of prey-derived nutrients. In this and many other host-associated systems, the diversity of the microbiota is predicted to influence rates of ecosystem flux. Using bacterial strains isolated from pitcher leaves at regular intervals over a one-year period, I tested whether the successional dynamics of pitcher leaves' natural bacterial source communities would affect the relationship between an experimental community's taxonomic richness and respiration rate. I determined the magnitude of this relationship to be unimodal with source community age. This positive richness effect on respiration was driven both by linear, additive species impacts and diffuse competition

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 can be time-dependent. Future studies on biodiversity-ecosystem function relationships are encouraged to adopt a dynamic species pool framework to increase the generalizability of their results.

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FIGURE LEGENDS

Figure 1. Conceptual diagram of species pool selection in biodiversity-ecosystem function experiments. Species pools are typically either chosen by sampling a community at a single point in time (a) or from species, 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).

Figure 2. Mean rates of carbon mineralization for synthetic bacterial communities were unimodal over time, independent of richness (R). Different letters within each richness level indicate significant differences between ages according to Tukey's range test ($p < 0.05$). Bars denote standard errors.

Figure 3. Relationships between strain richness and community respiration for synthetic bacterial communities assembled from pitchers of different ages. Black lines denote significant linear richness fits for individual communities within each age group ($p < 0.05$). Mean values for the response variables are presented for clarity. Bars denote standard error measurements.

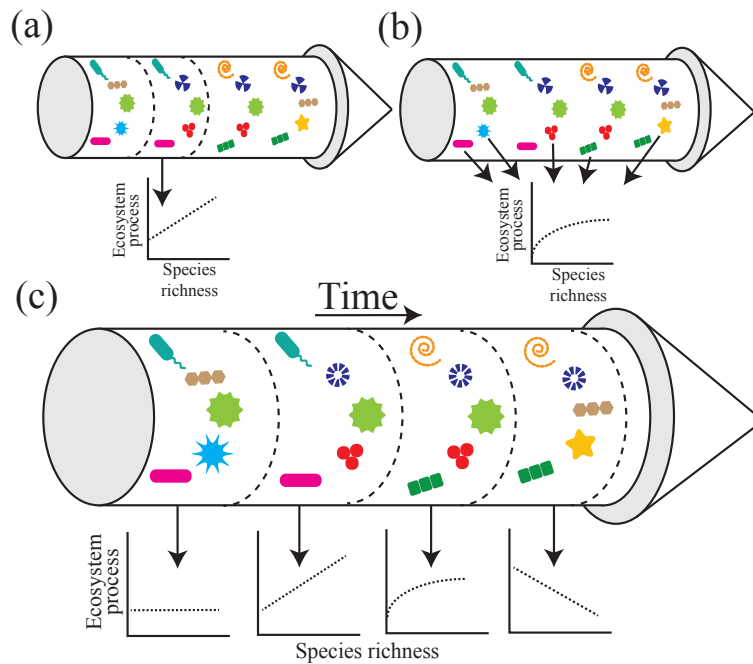
Figure 4. (a) Linear richness (β_{LR}) regression coefficients as a function of source community age. Bars denote 95% confidence intervals and shared letters between ages signify an overlap between the two estimates. Asterisks (*) denote coefficients found to be significantly greater than zero (F -test, $p < 0.05$). (b) Mean square estimates for the combined species impact (β_i) and nonlinear richness (β_{NLR}) parameters. These values represent the relative contributions of species-specific effects and species interactions, respectively, on rates of carbon mineralization once the effects of linear richness have been accounted for. Asterisks (*)

within points indicate that the coefficient's inclusion into the model provides a statistically improved fit over a null intercept-only model (χ^2 likelihood ratio test, $p < 0.05$).

Figure 5. Relative suppression of bacterial respiration in polycultures. Values of zero indicate that the sum of community members' respirations in their respective monocultures equaled the community's performance in polyculture. Values greater than zero indicate a greater suppression of potential respiration and may signify interspecific competitive or antagonistic interactions. Boxplots denote quartiles of raw data and white points are mean values. Letters shared by points within a richness group indicate that their means do not significantly differ from one another (Tukey's range test, $p < 0.05$).

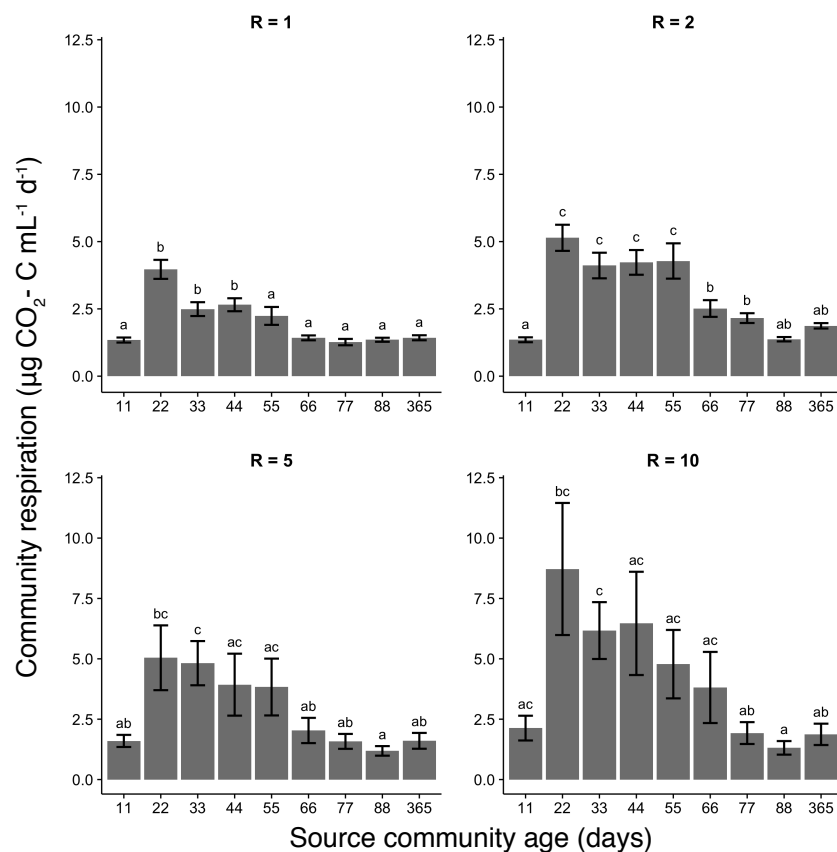
Figure 6. (a) The number of carbon substrates metabolized by each 10-species community on the Biolog GN2 microplate. (b) Jaccard distances between each community's carbon utilization profile plotted on first two principal coordinates. Colors represent source community ages as in (a).

728 **Figure 1.**



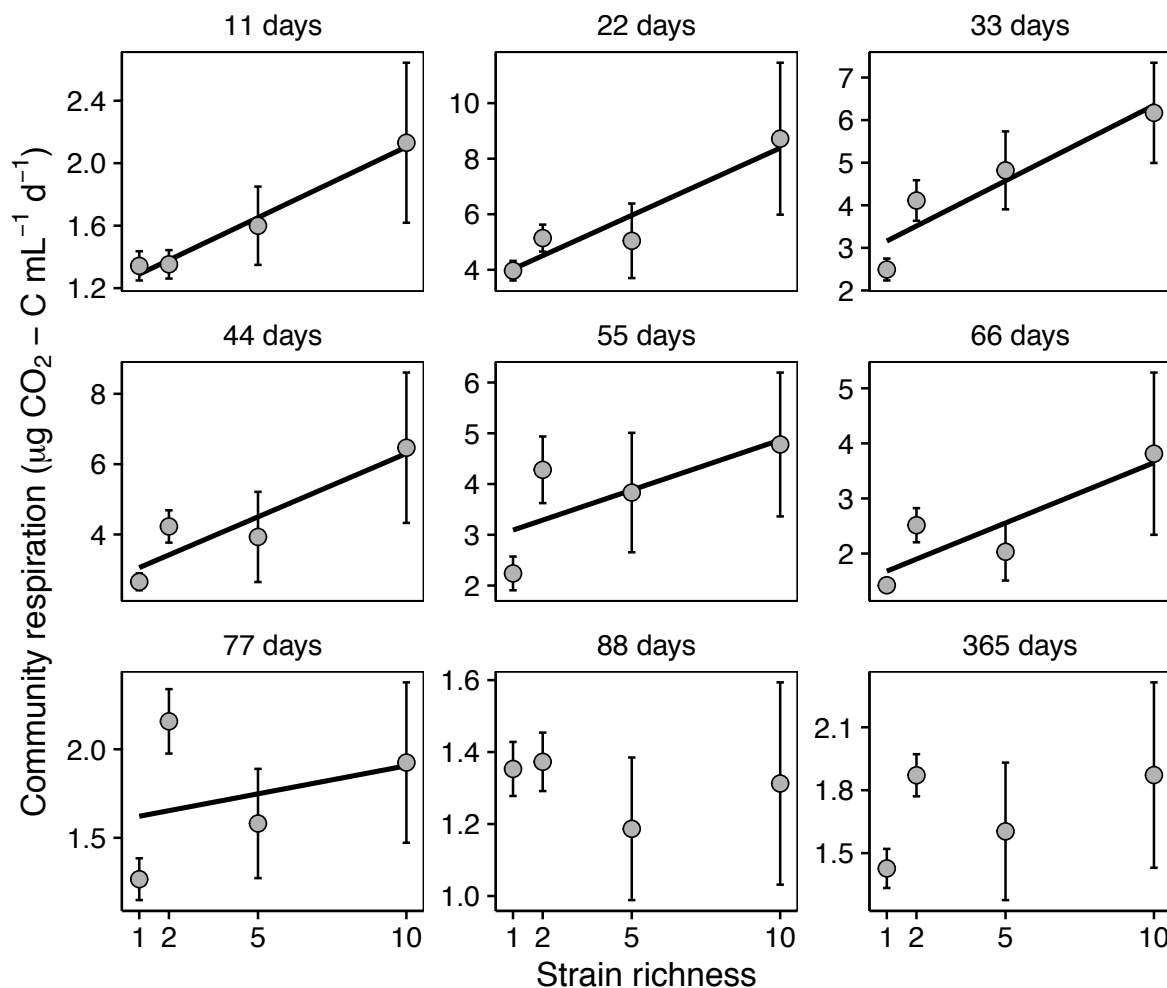
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730 **Figure 2.**



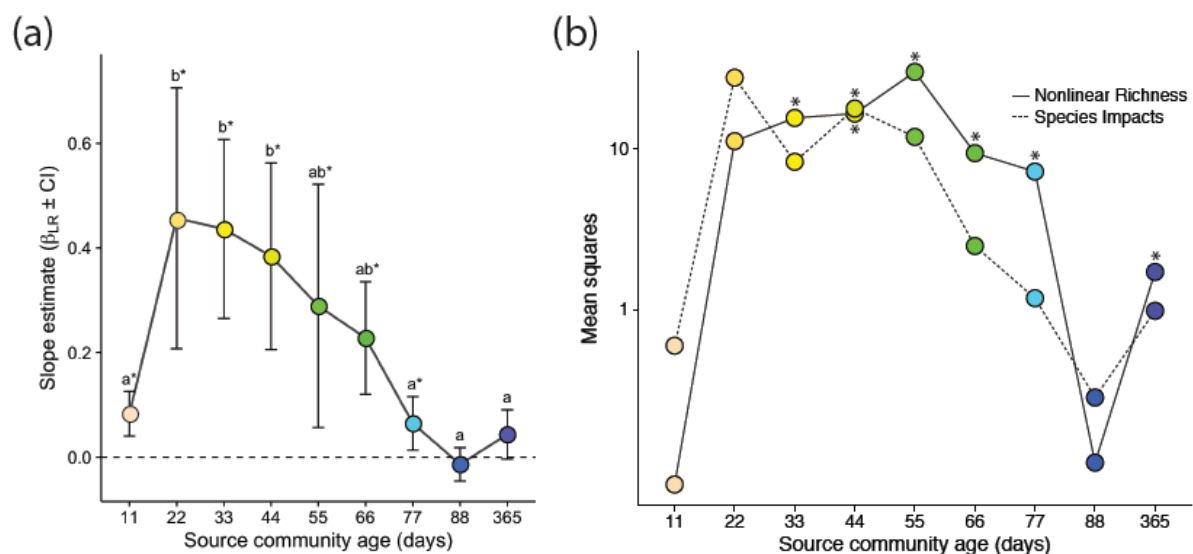
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732 **Figure 3.**



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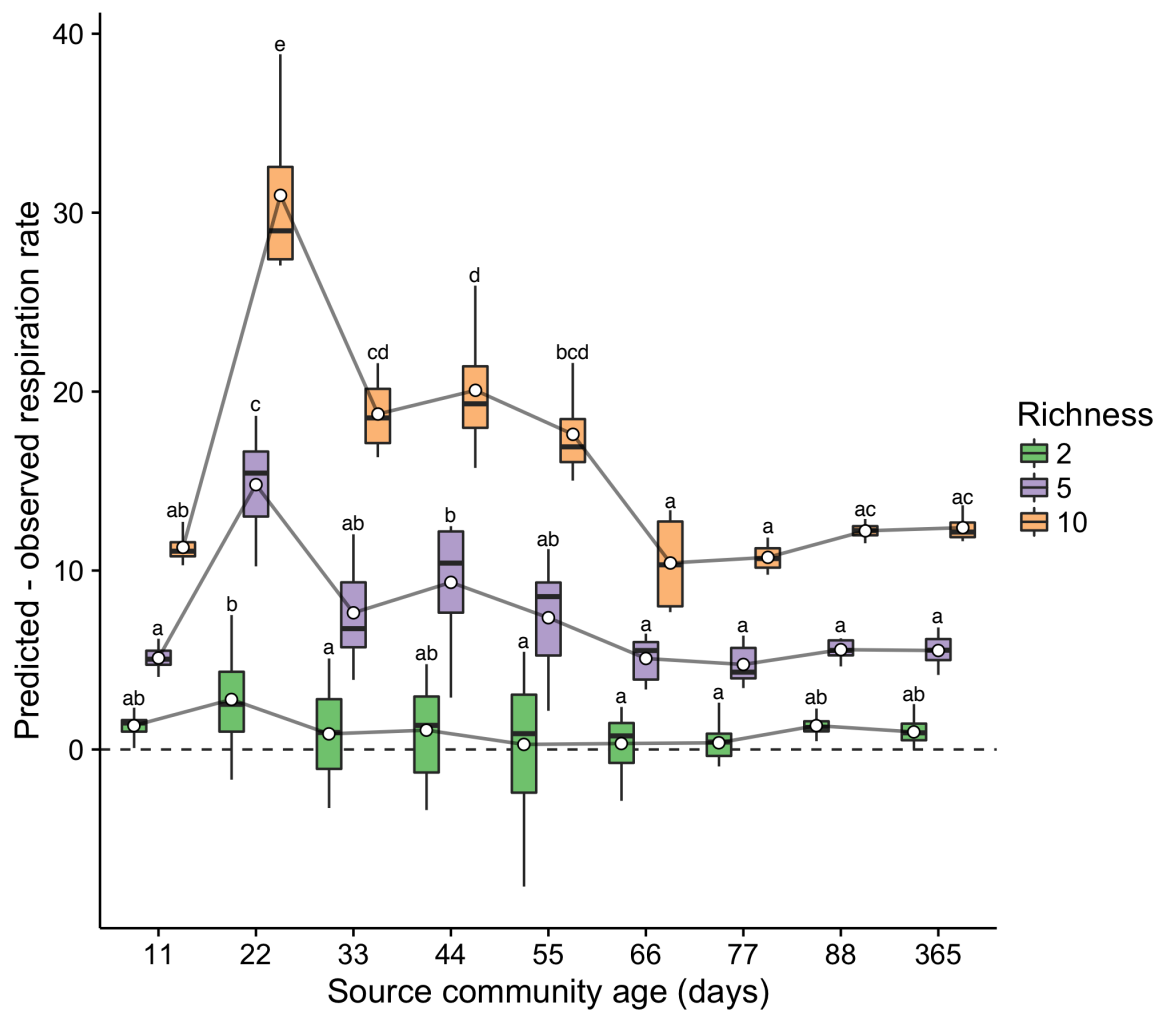
734 **Figure 4.**



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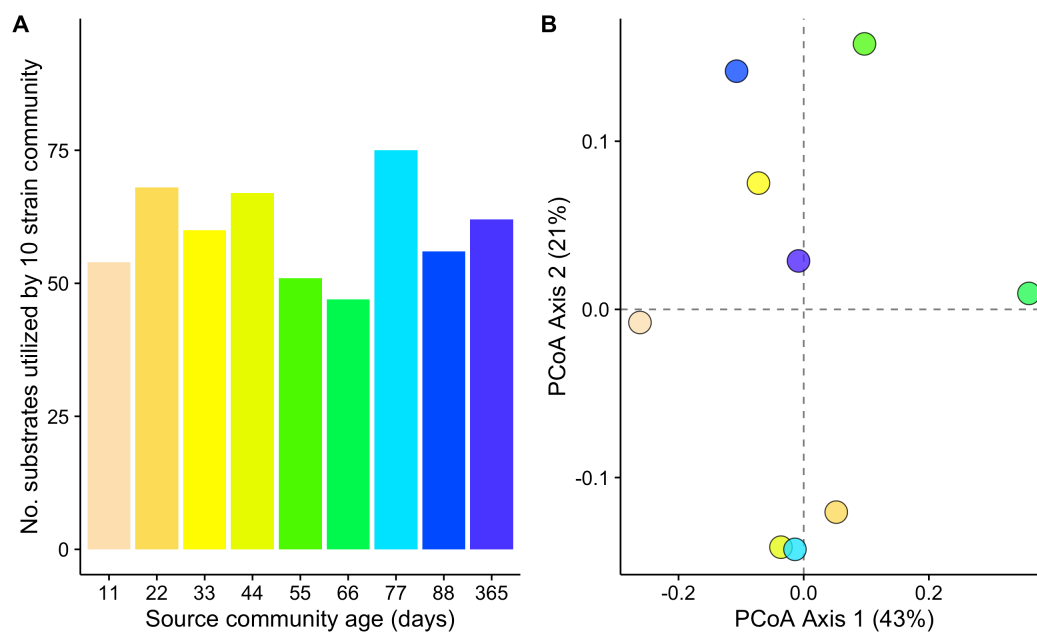
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737 **Figure 5.**



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739 **Figure 6.**



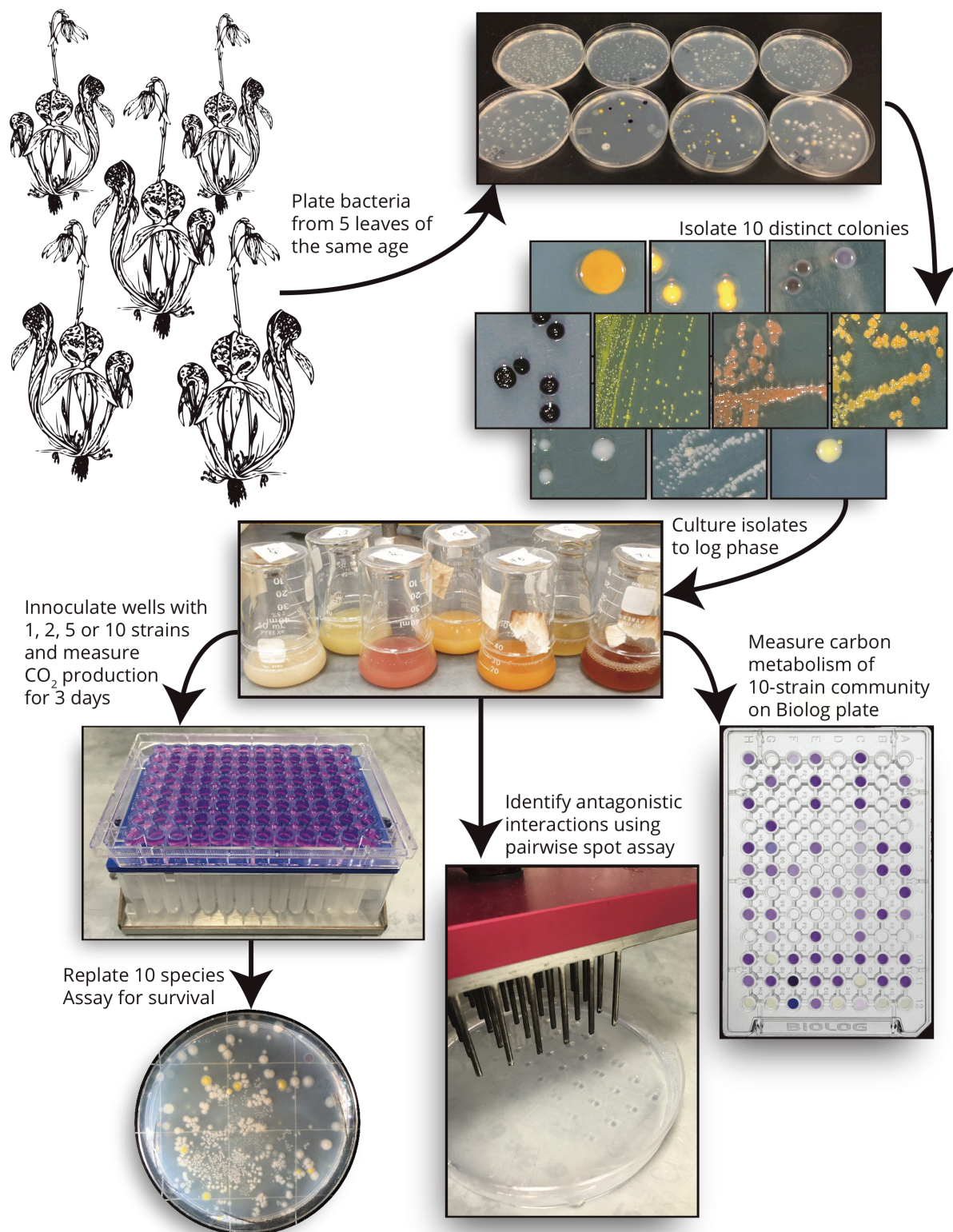
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Table 1. ANCOVA results for total respiration and respiration differences. Respiration rates were log-transformed to satisfy homoscedasticity. Richness and prey capture rates were treated as a continuous variables and age as a categorical variable with 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
Log respiration rate	Intercept	1	6.85	11.28	< 0.001	0.07
	Average leaf capture rate	1	16.58	27.32	< 0.001	
	Species richness	1	0.28	0.46	0.5	
	Interaction term	1	4.44	7.32	< 0.01	
	Residuals	1940	1177.32			
Response	Covariate	DF	SS	F	p	R ²
Expected - observed respiration	Intercept	1	689	154	< 0.001	0.89
	Source community age	8	116	3.3	< 0.01	
	Species richness	1	7480	1675.0	< 0.001	
	Interaction term	8	1133	31.7	< 0.001	
	Residuals	270	1206			

757 APPENDIX

758



759

Figure S1. Graphical flowchart of the experimental procedure. This process was repeated every 11 days until day 88 using the same pitcher leaves. Five additional year-old leaves were also sampled.

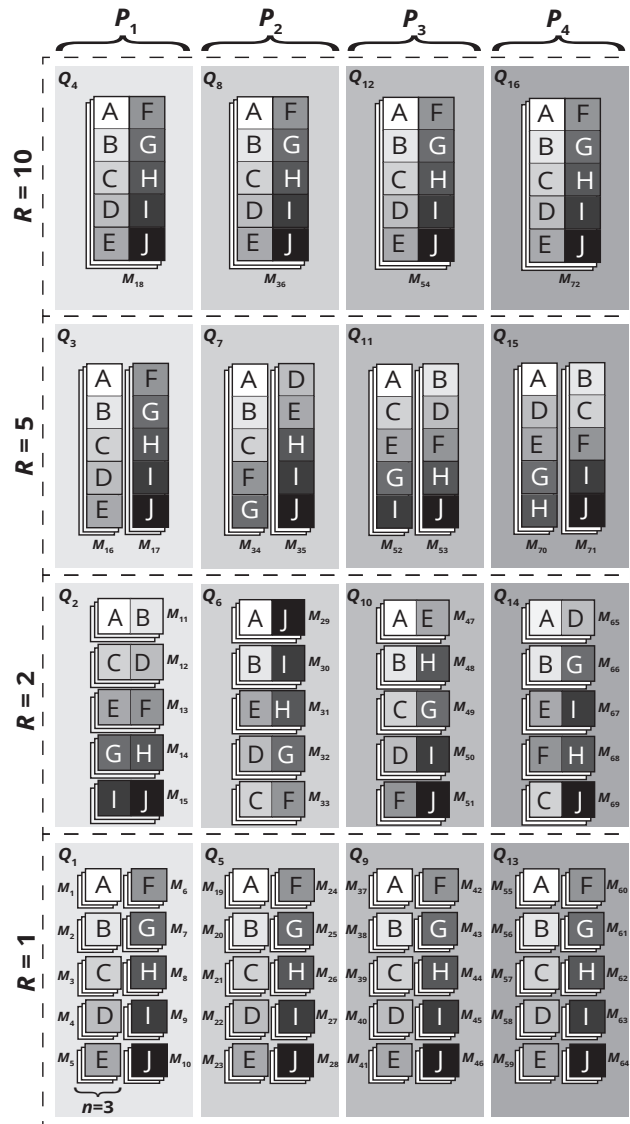


Figure S2. Diversity-ecosystem function experimental design, modified from (Bell *et al.* 2009). Each lettered box represents one of 10 individual bacterial strains isolated from a source pitcher leaves of a particular age. Each partition P represents a unique assignment of species to communities, M , where a species is chosen without replacement. These communities span 4 levels of species richness and are replicated three times per species pool Q . This design was used for each set of bacterial isolates from each source community age.

770 **Table S1.** Ordinary least squares estimates for parameters in equation 2 (see main text).

771 Rows labeled “ns” denote the effect was not statistically different from zero.

Age	Parameter		Mean	SE	<i>t</i>	<i>p</i>
11 days	Intercept	β_0	1.23	0.07	18.00	< 0.001
	Linear richness	β_{LR}	0.08	0.02	3.80	< 0.001
	Species effect	$\beta_i =$				
		β_1	-0.13	0.13	-0.96	ns
		β_2	0.12	0.13	0.91	ns
		β_3	0.05	0.13	0.36	ns
		β_4	0.02	0.13	0.18	ns
		β_5	0.18	0.13	1.40	ns
		β_6	0.07	0.13	0.54	ns
		β_7	0.17	0.13	1.33	ns
		β_8	-0.09	0.13	-0.73	ns
		β_9	-0.11	0.13	-0.83	ns
		β_{10}	-0.28	0.13	-2.14	< 0.05
	Nonlinear richness	$\beta_{NLR} =$				
		$R = 1$	0.03	0.06	0.41	ns
		$R = 2$	-0.05	0.09	-0.52	ns
		$R = 5$	-0.05	0.14	-0.34	ns
		$R = 10$	0.07	0.20	0.34	ns
Age	Parameter		Mean	SE	<i>t</i>	<i>p</i>
22 days	Intercept	β_0	3.66	0.40	9.16	< 0.001
	Linear richness	β_{LR}	0.46	0.13	3.61	< 0.001
	Species effect	$\beta_i =$				
		β_1	-0.02	0.77	-0.03	ns
		β_2	1.12	0.74	1.52	ns
		β_3	-1.13	0.74	-1.53	ns
		β_4	0.34	0.74	0.46	ns
		β_5	1.33	0.76	1.75	ns
		β_6	-0.81	0.75	-1.08	ns
		β_7	1.19	0.75	1.59	ns
		β_8	0.04	0.75	0.05	ns
		β_9	-0.98	0.75	-1.31	ns
		β_{10}	-1.07	0.77	-1.39	ns
	Nonlinear richness	$\beta_{NLR} =$				
		$R = 1$	-0.15	0.37	-0.41	ns
		$R = 2$	0.56	0.52	1.09	ns
		$R = 5$	-0.90	0.82	-1.10	ns
		$R = 10$	0.49	1.16	0.42	ns

774 **Table S1**, continued

Age	Parameter		Mean	SE	<i>t</i>	<i>p</i>
33 days	Intercept	β_0	2.44	0.27	8.88	< 0.001
	Linear richness	β_{LR}	0.44	0.09	5.03	< 0.001
	Species effect	$\beta_i =$				
		β_1	-0.13	0.53	-0.25	ns
		β_2	-0.10	0.51	-0.20	ns
		β_3	-0.23	0.51	-0.45	ns
		β_4	0.14	0.52	0.28	ns
		β_5	0.20	0.53	0.37	ns
		β_6	-0.11	0.52	-0.21	ns
		β_7	1.28	0.52	2.46	< 0.05
		β_8	-0.57	0.52	-1.09	ns
		β_9	0.27	0.52	0.51	ns
		β_{10}	-0.75	0.53	-1.40	ns
	Nonlinear richness	$\beta_{NLR} =$				
		$R = 1$	-0.38	0.25	-1.51	ns
		$R = 2$	0.80	0.36	2.26	< 0.05
		$R = 5$	0.20	0.56	0.36	ns
		$R = 10$	-0.63	0.80	-0.79	ns

Age	Parameter		Mean	SE	<i>t</i>	<i>p</i>
44 days	Intercept	β_0	2.59	0.29	9.06	< 0.001
	Linear richness	β_{LR}	0.38	0.09	4.25	< 0.001
	Species effect	$\beta_i =$				
		β_1	0.66	0.54	1.21	ns
		β_2	0.48	0.52	0.92	ns
		β_3	-0.86	0.52	-1.65	ns
		β_4	-0.62	0.52	-1.19	ns
		β_5	0.90	0.54	1.67	ns
		β_6	-0.27	0.53	-0.51	ns
		β_7	1.22	0.53	2.31	< 0.05
		β_8	-0.60	0.53	-1.14	ns
		β_9	-0.16	0.53	-0.30	ns
		β_{10}	-0.74	0.54	-1.37	ns
	Nonlinear richness	$\beta_{NLR} =$				
		$R = 1$	-0.32	0.26	-1.25	ns
		$R = 2$	0.87	0.36	2.40	<0.05
		$R = 5$	-0.58	0.57	-1.02	ns
		$R = 10$	0.03	0.81	0.04	ns

778 **Table S1**, continued

Age	Parameter		Mean	SE	<i>t</i>	<i>p</i>
55 days	Intercept	β_0	2.48	0.29	9.06	< 0.001
	Linear richness	β_{LR}	0.29	0.09	4.25	< 0.05
	Species effect	$\beta_i =$				
		β_1	0.18	0.74	0.25	ns
		β_2	-1.00	0.72	-1.38	ns
		β_3	-0.47	0.71	-0.67	ns
		β_4	0.68	0.70	0.96	ns
		β_5	0.19	0.72	0.26	ns
		β_6	0.89	0.71	1.26	ns
		β_7	0.71	0.71	1.00	ns
		β_8	-0.71	0.71	-1.00	ns
		β_9	0.33	0.69	0.47	ns
		β_{10}	-0.83	0.77	-1.08	ns
	Nonlinear richness	$\beta_{NLR} =$				
		$R = 1$	-0.53	0.34	-1.54	ns
		$R = 2$	1.17	0.49	2.40	< 0.05
		$R = 5$	-0.08	0.77	-0.10	ns
		$R = 10$	-0.56	1.09	-0.52	ns

Age	Parameter		Mean	SE	<i>t</i>	<i>p</i>
66 days	Intercept	β_0	1.42	0.17	8.23	< 0.001
	Linear richness	β_{LR}	0.23	0.06	4.17	< 0.001
	Species effect	$\beta_i =$				
		β_1	-0.03	0.34	-0.08	ns
		β_2	-0.05	0.33	-0.15	ns
		β_3	-0.06	0.33	-0.18	ns
		β_4	-0.40	0.33	-1.22	ns
		β_5	0.34	0.34	1.01	ns
		β_6	0.65	0.33	1.95	ns
		β_7	0.19	0.33	0.59	ns
		β_8	-0.12	0.33	-0.36	ns
		β_9	-0.13	0.33	-0.38	ns
		β_{10}	-0.40	0.34	-1.18	ns
	Nonlinear richness	$\beta_{NLR} =$				
		$R = 1$	-0.23	0.16	-1.42	ns
		$R = 2$	0.64	0.22	2.86	< 0.01
		$R = 5$	-0.53	0.35	-1.49	ns
		$R = 10$	0.11	0.50	0.23	ns

782 **Table S1**, continued

Age	Parameter		Mean	SE	<i>t</i>	<i>p</i>
77 days	Intercept	β_0	0.14	0.08	17.49	< 0.001
	Linear richness	β_{LR}	0.07	0.03	2.48	< 0.05
	Species effect	$\beta_i =$				
		β_1	-0.12	0.16	-0.76	ns
		β_2	-0.01	0.15	-0.06	ns
		β_3	-0.01	0.15	-0.06	ns
		β_4	-0.20	0.15	-1.29	ns
		β_5	0.36	0.16	2.27	< 0.5
		β_6	0.28	0.15	1.83	ns
		β_7	0.14	0.15	0.88	ns
		β_8	-0.06	0.15	-0.41	ns
		β_9	0.01	0.15	0.09	ns
		β_{10}	-0.39	0.16	-2.46	< 0.5
	Nonlinear richness	$\beta_{NLR} =$				
		$R = 1$	-0.24	0.07	-3.52	<0.001
		$R = 2$	0.59	0.10	6.08	<0.001

783

Age	Parameter		Mean	SE	<i>t</i>	<i>p</i>
88 days	Intercept	β_0	0.14	0.05	26.57	< 0.001
	Linear richness	β_{LR}	-0.01	0.02	-0.85	ns
	Species effect	$\beta_i =$				
		β_1	-0.04	0.10	-0.41	ns
		β_2	0.06	0.10	0.58	ns
		β_3	0.13	0.10	1.30	ns
		β_4	-0.13	0.10	-1.32	ns
		β_5	0.16	0.10	1.66	ns
		β_6	0.08	0.10	0.78	ns
		β_7	-0.07	0.10	-0.72	ns
		β_8	0.03	0.10	0.33	ns
		β_9	-0.02	0.10	-0.21	ns
		β_{10}	-0.20	0.10	-1.94	ns
	Nonlinear richness	$\beta_{NLR} =$				
		$R = 1$	0.00	0.05	-0.04	ns
		$R = 2$	0.03	0.07	0.47	ns

784

785

786 **Table S1**, continued

Age	Parameter		Mean	SE	<i>t</i>	<i>p</i>
365 days	Intercept	β_0	1.50	0.08	19.75	< 0.001
	Linear richness	β_{LR}	0.04	0.02	1.81	ns
	Species effect	$\beta_i =$				
		β_1	-0.11	0.15	-0.73	ns
		β_2	0.30	0.14	2.16	ns
		β_3	0.11	0.14	0.75	ns
		β_4	-0.18	0.14	-1.26	ns
		β_5	0.30	0.14	2.05	ns
		β_6	-0.06	0.14	-0.42	ns
		β_7	0.09	0.14	0.66	ns
		β_8	-0.02	0.14	-0.16	ns
		β_9	-0.22	0.14	-1.58	ns
		β_{10}	-0.21	0.15	-1.43	ns
	Nonlinear richness	$\beta_{NLR} =$				
		$R = 1$	-0.11	0.07	-1.69	ns
		$R = 2$	0.29	0.10	2.97	< 0.01
		$R = 5$	-0.11	0.15	-0.73	ns
		$R = 10$	-0.06	0.22	-0.28	ns

787