Resource pulses can alleviate the biodiversity–invasion relationship in soil microbial communities

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Abstract. The roles of species richness, resource use, and resource availability are central to many hypotheses explaining the diversity–invasion phenomenon but are generally not investigated together. Here, we created a large diversity gradient of soil microbial communities by either assembling communities of pure bacterial strains or removing the diversity of a natural soil. Using data on the resource-use capacities of the soil communities and an invader that were gathered from 71 carbon sources, we quantified the niches available to both constituents by using the metrics community niche and remaining niche available to the invader. A strong positive relationship between species richness and community niche across both experiments indicated the presence of resource complementarity. Moreover, community niche and the remaining niche available to the invader predicted invader abundance well. This suggested that increased competition in communities of higher diversity limits community invasibility and underscored the importance of resource availability as a key mechanism through which diversity hinders invasions. As a proof of principle, we subjected selected invaded communities to a resource pulse, which progressively uncoupled the link between soil microbial diversity and invasion and allowed the invader to rebound after nearly being eliminated in some communities. Our results thus show that (1) resource competition suppresses invasion, (2) biodiversity increases resource competition and decreases invasion through niche preemption, and (3) resource pulses that cannot be fully used, even by diverse communities, are favorable to invasion.

Key words: assemblage experiment; biological invasion; community niche; diversity–invasion effect; removal experiment; resource availability; resource pulse; resource-use mechanism.

Introduction

Invasive species have the potential to alter native community structure and affect the functioning of ecosystems (Hooper et al. 2005). The extent of this impact is tightly linked to the level of biodiversity at a local scale, which plays a key role in buffering a system from invasion (Naeem et al. 2000, Kennedy et al. 2002, Zavaleta and Hulvey 2004). There is no general theory that ties together all the factors influencing what is colloquially known as the diversity–invasion relationship, whereby more diverse communities resist invasion better than less diverse communities. Still, most studies revolve around the role of resource-use mechanisms, whether it be competition between the resident community and the invader or the availability of resources in the environment (Catford et al. 2009). Niche theory purports that the probability of an invasion will exponentially decrease as diversity increases due to the increased partitioning of resources among more diverse

communities (Tilman 2004). In line with this theory, a general and emerging principle to consider when investigating biological invasion is that resident communities should resist invasion when their resource uptake best matches resource supply (i.e., the fluctuating resource hypothesis; Davis et al. 2000). Should resource supply outpace resource uptake (due to the amount or forms of resources supplied), the chance of invasion should increase. Moreover, the theories proposed by both Davis et al. (2000) and Tilman (2004) predict that resource pulses will make a community more invasible and facilitate coexistence between an invader and the community. These theories have been supported by empirical examples coming from macroorganisms, and resource pulses have also been shown to further depend on their timing, as well as the life history and uptake capabilities of the invader and resident community members (Davis and Pelsor 2001, Renne et al. 2006, Li and Stevens 2012). Interestingly, even though the fluctuating resource hypothesis (FRH) is rooted in the idea that the degree of competition is negatively related to the amount of unused resources, the FRH predicts the absence of any diversity–invasion effect due to the

assumption that a resource pulse will suppress competition among the resident and invader populations (Davis et al. 2000). While this is logical, never before has this prediction been tested and demonstrated.

Invasion patterns from microbial communities indicate that such communities display diversity–invasion relationships that are similar to those of higher organisms when measured on a local scale (van Elsas et al. 2007, 2012, Jousset et al. 2011, Eisenhauer et al. 2013, Vivant et al. 2013). Indeed, reducing the diversity of natural soil communities has shown to increase the survival time of invading *Escherichia coli* and *Listeria monocytogenes* (van Elsas et al. 2012, Vivant et al. 2013). Experiments creating diversity gradients with simplified, assembled microbial communities have shown similar patterns. For instance, communities containing an increasing number (from one to eight strains) of *Pseudomonas fluorescens* genotypes increasingly resisted invasion from *Pseudomonas putida* and *Serratia liquefaciens* (Jousset et al. 2011, Eisenhauer et al. 2013).

Harnessing the capability to manipulate and create microbial communities provides an ideal opportunity to foster our understanding of the mechanisms controlling the diversity–invasion relationship. Microbial assemblage experiments using simplified communities have touched upon the mechanisms that make more diverse communities more resistant to invasion than lower-diversity communities. For instance, by assessing the impact of the pathogenic bacterium *Ralstonia solanacearum* on tomato plants in soil microcosms containing five, ten, or fifteen rhizobacterial strains, Irikiin et al. (2006) showed that higher frequencies of diseased plants were found in communities of lower diversity and lower resource-use potential. Thus, the large competitive effects in the more diverse communities could have limited the pathogen's growth, survival, and infection capability. In another experiment, van Elsas et al. (2012) showed that the competitive effects of richness limited the short-term impact of the invader on community functioning, measured as their ability to use carbon substrates. Moreover, the importance of the community's functional diversity among bacterial communities in conjunction with the number of resources available (i.e., niche dimensionality) has highlighted the importance of resource complementarity in invasion resistance (Eisenhauer et al. 2013). Taken together, these studies encompass the most basic mechanistic understanding we have of the diversity–invasion relationship. In short, more diverse communities better exploit resources; this stems the invader's access to vital resources and results in its eventual death. However, this mechanism has not been explored throughout more realistic levels of bacterial species diversity. Moreover, from an invader point of view, never before has its actual resource availability been quantified across a diversity gradient, nor have the effects of a resource pulse been examined to understand the extent to which it could permit invasion across a diversity gradient.

We focus on a three-step argumentation to set forth the extent to which resource availability influences the diversity–invasibility relationship for soil microbial communities. Our arguments rely on the assumptions that (1) resource competition suppresses invasion, (2) diversity increases resource competition due to niche preemption, and (3) resource pulses can suppress competition and therefore promote invasibility even in highly diverse communities. Specifically, we test the hypothesis that higher levels of resident microbial diversity reduce the niche available to an invader via increased competitive interactions and that this relationship is alleviated by the application of a resource pulse. As explained in Fig. 1, we propose that highly diverse communities should better exploit available resources than less diverse ones. In low resource environments, high diversity levels thus limit the availability of scarce resources for the invader and result in a fast decline of invader abundance (Fig. 1A). In high resource environments (Fig. 1B), although more diverse communities will still use more resources than less diverse ones, ample resources should weaken competition, leading to high invader abundances in both high- and low-diversity communities. The addition of resources should increase the success of an invader, even in highly diverse communities under low resources where the invader would otherwise be excluded (Fig. 1C).

We tested these hypotheses by manipulating the diversity of soil microbial communities and subjecting them to invasion by a nonpathogenic derivative of *E. coli* O157:H7. We constructed a large gradient of diversity via assemblage and removal approaches. Pure bacterial strains were assembled into communities and inoculated into sterile soil (assemblage). Natural soil was diluted and used as an inoculum for sterile soil (removal). All constructed microbial communities were then subjected to invasion. By using a large array of resources to separately quantify the community's and invader's resource-use capacities, we used the community niche metric, which has previously been used to predict diversity–ecosystem functioning relationships (Salles et al. 2009, 2012), to calculate the niche breadth of the community and the extent of resource complementarity. Also using the resource-use capacity data, we quantified the remaining niche of the invader (i.e., the resources unused by the resident community, which are suitable substrates for the invader), which were used to predict an invader's survival. To complement the aforementioned approaches, a direct test of the potential importance of competition for resources was performed by manipulating resource availability during the invasion process. We tested whether a resource pulse could suppress the effects of diversity and competition on the invader's survival, which was achieved by subjecting selected communities from the assemblage experiment to a D-galactose pulse 105 days after *E. coli* invasion, when the effects of competition had already been clearly seen.
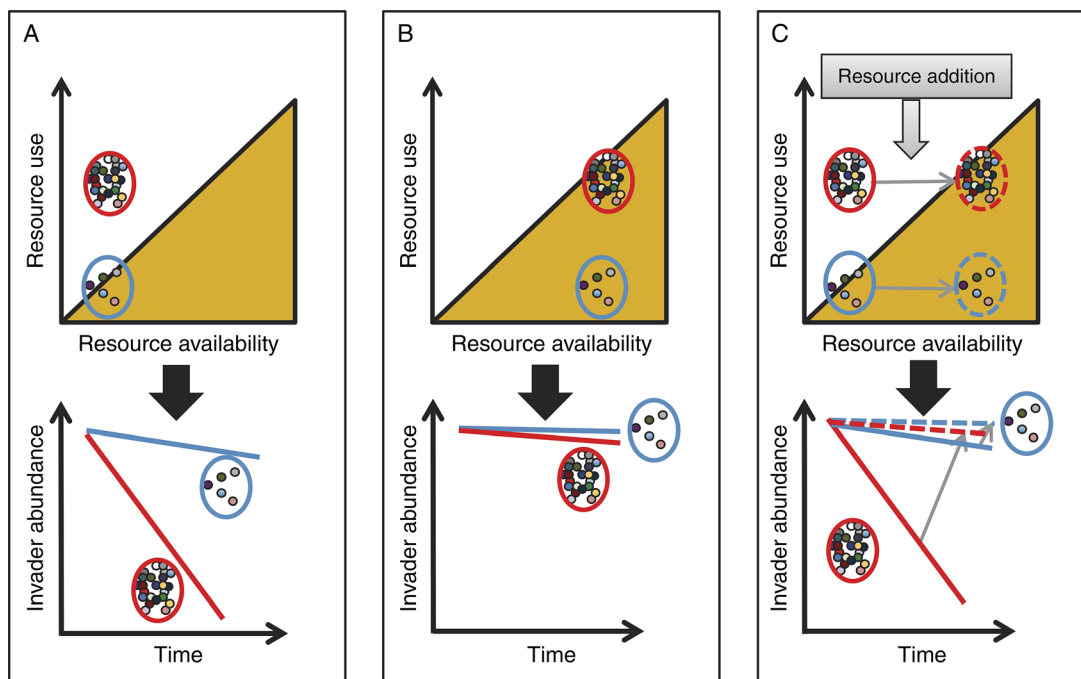
Fig. 1. Theoretical representation of an invader's survival as dependent upon resource availability and use by the resident community. (A) When resources are limited, more diverse communities will reduce the survival of an invader better than less diverse communities because they better exploit resources, allowing niche preemption. (B) When resources are readily available, the invader's survival time will increase and community diversity will be less important in determining invasion success. (C) If resources are added to invaded communities under a low resource environment, resource competition will be alleviated. As such, the survival time of the invader will increase, particularly for highly diverse communities. Blue circles and lines represent a species-poor community and the corresponding temporal variation of an invader's survival; red circles and lines represent the same for a highly diverse community. The orange-shaded portion of each graph represents the plane where a community is susceptible to invasion. The gray arrows and dashed blue and red lines represent a shift and the new state of communities after a resource pulse. Thus, communities that had been low in available resources now have a high resource availability, shifting them into the orange-shaded portion that indicates communities are highly susceptible to invasion. In turn, the abundance of any invader in these communities should also increase.

METHODS

Soil and microcosms

The sandy loam grassland soil from the sampling site Wildekamp, The Netherlands (51°59′38.45″ N, 5°39′58.72″ E) was used as the sterile soil matrix for the assemblage and removal experiments and as the inoculum for the removal experiment. Soil was sieved through a 4-mm mesh, homogenized, and sterilized via gamma irradiation (50 kGy) in sealed plastic bags. Fifty grams of sterile soil were then aseptically transferred into sterile glass microcosms capped with sterile aluminum foil. The soil pH of each microcosm was adjusted to pH 7 via addition of $Ca(OH)_2$, and the water content of the soil was brought to 50% of the soil water holding capacity (WHC) with sterile water. Water content was kept constant by replenishing with sterile water until the inoculation of communities.

Assemblage experiment: setup and design

A large pool (>100) of bacterial colonies were isolated from eight Dutch agricultural soils. Isolation was carried out in 1:10 serial dilutions of soil, whereby the 10^{-4} dilution was plated onto trypticase soy agar (TSA; BD, Heidelberg, Germany) and incubated for three days at 23°C. After incubation, colonies were picked on the basis of different morphologies and restreaked at least twice to ensure purity. Isolated strains were stored at −80°C in 25% glycerol. All strains were screened using the repetitive BOX polymerase chain reaction (PCR) method (Bathe et al. 2006), giving rise to 75 different bacterial strains.

In order to create the communities, all 75 strains were separately grown on TSA at 23°C for 3 d. Cells were then harvested from plates with 0.8% NaCl via plate washing, and the optical density (OD) for each strain was adjusted to $OD_{600} = 1$. Strains were then assembled in an equal volume (i.e., at maximal evenness) into community master mixes and used to inoculate microcosms. One milliliter of each community master mix or sterile water (for sterile treatment) was added to microcosms, bringing the soil moisture to 65% of the WHC. Communities were assembled according to a broken stick design to create communities of 5, 15, and 30 bacterial species (Bell et al. 2009; see table of communities in Appendix A). The broken stick method

was chosen because it maximizes the separation of richness and compositional effects due to its nested approach. We created 10 communities of 30 species; 12 of 15 species; 24 of 5 species; and a sterile soil control (considered as 0 species, replicated thrice). Microcosms were left for 60 d to allow bacterial abundances to stabilize, upon which the invader was introduced. Soil moisture content was checked weekly and replenished with sterile water to maintain a WHC of 65%. Five destructive sampling campaigns were used for each community: days 0, 11, 44, 75, and 95. This comprised a total of 245 microcosms.

Removal experiment: setup and design

For the removal approach, community inoculums were prepared by making an initial 1:2, soil : water mixture of natural (nonsterile) Wildekamp soil. This solution was then 1:10 serially diluted in sterile water up to the 10^{-6} dilution, and the 10^{-1}, 10^{-3}, and 10^{-6} dilutions were inoculated into sterilized Wildekamp soil to create a diversity gradient. Each microcosm contained 50 g of soil and received 2.9 mL of soil dilution or sterile water, increasing the soil water content to 65% of the WHC. The four diversity treatments were thus: 10^{-1}, 10^{-3}, 10^{-6}, and sterile soil. Microcosms were incubated for 79 d in order to establish the same soil bacterial abundances across treatments. The microcosms were weighed weekly and replenished with sterile water to maintain the soil moisture at 65% of the WHC. Six destructive sampling campaigns were used at days 0, 5, 11, 18, 28, and 75 using three replicates for each treatment and each date. This comprised a total of 72 microcosms.

Quantification of bacterial abundances and initial species richness

In both experiments, total cultural bacteria were checked before the introduction of the invader to assure comparable numbers of cells/g of soil in all treatments. Total bacteria were enumerated by 1:10 serial dilution plating on TSA medium and incubated for 4 d at 28°C. In the removal experiment, in order to account for unculturable bacteria, the V5–V6 variable region of the 16S rRNA gene was also quantified via quantitative PCR and extracted soil DNA from day 0 was used as a template (see Appendix B for detailed protocol).

In the assemblage experiment, estimations of species richness were taken as the number of species initially inoculated into the community (e.g., 0, 5, 15, or 30 species). In the removal experiment, initial bacterial species richness was quantified by 454 pyrosequencing of the 16S rRNA gene, using observed operational taxonomic units (OTUs) at a sequence similarity of 97% (see Appendix C for detailed protocol). For simplicity we refer to OTUs as species, but this is only an operational definition and may not represent the actual number of species in the soil. In order to mitigate this problem and show that estimations of species

richness are reliable between diversity treatments, we have provided rarefaction curves of sequencing data (Appendix D).

Invader introduction and enumeration

E. coli O157:H7 derivative strain T, which has been used in previous studies to examine the diversity–invasion relationship (van Elsas et al. 2007, 2012), was introduced with sterile water into the microcosms at a level of 5×10^7 cells/g soil. Indeed, *E. coli* invading soil ecosystems, such as from the excrements from cattle, can reach initial and total population sizes of up to 10^9 cells (Horton et al. 2011). Since our case aims to portray how *E. coli* survives in soil ecosystems, we used a moderate initial population size. If one considers the soil to have total bacterial population of $\sim10^9$ cells/g soil, and we add 5×10^7 invader cells/g soil, then the invading organism was outnumbered on a level of $\sim$19 native cells for 1 invading cell, representing a gross mismatch for colonizing space in the soil. Introduction of *E. coli* raised the soil moisture to 75% of its WHC. Upon inoculation of the invader, the soil moisture was checked weekly and maintained at 75% of WHC with sterile water until the end of the experiment. The invader, which harbors resistances to rifampicin and kanamycin, was enumerated on each sampling day via plating on trypticase soy agar supplemented with said antibiotics at concentrations of 10 and 50 μg/mL, respectively. Plates were incubated at 37°C for 24 h.

Quantification of soil community niche and remaining niche available to the invader

The resource-use capacities of all the 75 strains used in the assemblage experiment and of the invader were screened on the Biolog GEN III microtiter plate, which contains 71 different carbon sources (Biolog, Haward, California, USA). Omnilogs units that measure the activity of the carbon sources are generated by the Biolog Omnilog software and are an absorbance value calculated from readings of color intensity. Fresh strains were grown on TSA plates and then inoculated onto the GEN III plate according to manufacturer's protocol with accompanying inoculation fluid B. Inoculated plates were monitored in Biolog's omnilog machine for 48 h at 23°C with measurements taken every 15 min. Raw data were normalized (by the maximum value observed across all strains) and used to calculate the community niche for each assembled community, according to Salles et al. (2009). Briefly, from the resource-use capacities of our 75 strains, the highest level of use observed for each of the 71 carbon sources among all strains in a respective community were summed to calculate the community niche. The relationship between *E. coli* abundance and the assembled community niche was tested by regression (data were log-transformed, if needed).

In the removal experiment, the resource-use capacity of each soil community was directly measured at day 0

by inoculating the soil community onto a GEN III plate (see Appendix E for detailed protocol). Data for each Biolog plate were normalized (independently by the maximum value for each plate).

For both experiments, the remaining niche available to the invader was calculated by subtracting the community from the invader resource-use capacities for each of the 71 carbon sources. Negative values indicated competitive superiority by the community and positive values the reverse. The positive values were summed and taken as a proxy of the remaining niche available to the invader. In short, we examined the overlap between invader and community resource-use profiles over an array of 71 carbon sources. Across all resources, values indicating an advantage for the invader were summed and taken as a proxy for the remaining niche available to the invader.

Resource pulse experiment

In order to further test the role of resource availability as a limiting factor in *E. coli*'s survival, we added D-galactose 105 d after invader inoculation to a selected group of assembled microcosms that had varied abilities to use this carbon source, from no use to a relatively strong use (3 communities of 30 species, 4 for 15 species, 6 for 5 species, and 2 for sterile soil). The invader was capable of using D-galactose as a substrate. The remaining soil (around 35 g total) of each flask that was sampled at day 95 was aseptically split into two sterile 50-mL Falcon tubes (BD Biosciences, Bedford, Massachusetts, USA), one for the addition of carbon, one for the addition of water that acted as a control. D-galactose was added to each flask in the carbon treatment group at a concentration of 6.25 mg/g soil. This slightly raised the soil moisture to 77.9% of the WHC. Control flasks were given an equal amount of water. *E. coli* was then enumerated 2, 5, and 10 d after the D-galactose pulse (in both amended and control treatments) as described in *Methods: Invader introduction and enumeration*.

Data analysis and statistics

All statistical tests were performed in SPSS (IBM 2011). One-way analysis of variance (ANOVA) tests were used to examine potential differences of total culturable bacteria and 16S rRNA gene copies between richness treatments in each experiment. Two-way ANOVA tests with interaction were used to assess *E. coli* survival as affected by time (sampling day) and initial species richness. Tukey's post hoc accompanied ANOVA analyses to indicate potential pairwise differences between richness treatments. Pearson correlations were used to examine the potential relationships between invader abundance and (1) microbial community richness, (2) community niche, (3) remaining niche availability, and (4) D-galactose use by the soil community. In the latter case, the relationship between D-galactose use and the invader's regrowth, we exam-

ined the bacterial resource-use profiles of individual strains to extract the maximum value of D-galactose use among each community and used it to predict invader abundance after the resource pulse. Relationships between microbial community richness and community niche were also analyzed using Pearson correlations. 16S rRNA gene amplicon sequences generated by pyrosequencing were processed using Quantitative Insights Into Microbial Ecology (QIIME) version 6.0 toolkit (Caporaso et al. 2010). Species richness was calculated in QIIME as the observed number of OTUs per sample (see Appendix F for detailed protocol).

Results

Total bacterial abundances

Total culturable bacterial counts indicated that soil bacterial abundances were not significantly different between treatments before the introduction of the invader in both experiments (ANOVA: assemblage experiment, $F_{2,43} = 0.135$, $P = 0.88$; removal experiment, $F_{2,3} = 1.121$, $P = 0.43$). In the assemblage experiment, the mean of total bacteria across all treatments was 5.35×10^8 cells/g soil, ranging from 4.4×10^6 to 6.5×10^9 cells/g soil. In the removal experiment, the mean of total cultural bacteria across treatments was 1.36×10^8 cells/g soil, ranging from 4.65×10^7 to 4.25×10^8 cells/g soil. Also in the removal experiment, where nonculturable bacteria likely represented most of the soil bacterial population, the mean number of 16S rRNA gene copies assessed by quantitative PCR was 7.69×10^{11} copies/g soil, ranging from 4.00×10^{11} to 1.09×10^{12} copies/g soil. Although the number of 16S rRNA gene copies was slightly higher in the 10^{-6} treatment compared to the 10^{-1} and 10^{-3} treatments, copy number did not significantly differ between treatments (ANOVA, $F_{2,9} = 4.377$, $P = 0.07$).

Invader survival according to time and species richness

In the assemblage experiment, a progressive decline in *E. coli* population densities, as measured by colony-forming unit (CFU) counts, was observed throughout the experiment, and this decline was significantly affected by time, species richness, and the interaction between these two factors (Fig. 2A, Table 1). The overall effect of time significantly reduced *E. coli* population densities at each sampling day (Tukey's test, $P < 0.05$). The overall effects of species richness on *E. coli* abundance was shown to be due to differences, on the one hand, between the sterile soil treatment and species richness treatments and, on the other hand, between the species richness treatments. Specifically, *E. coli* abundance was significantly higher in the sterile soil treatment when compared with all other treatments (Tukey's test, $P < 0.05$; see Fig. 2A, B). *E. coli* abundance was slightly but significantly higher in the 5 species treatment as compared to the 15 (Tukey's test, $P = 0.022$) and 30 species treatments (Tukey's test, $P = $
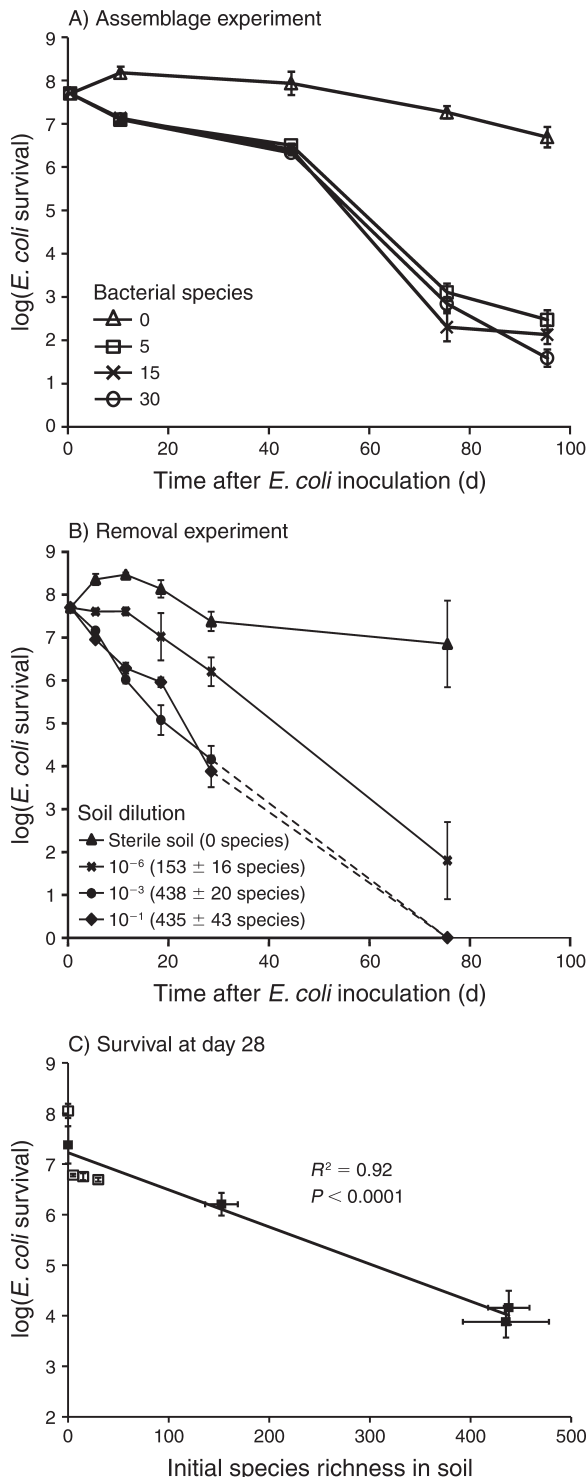
A) Assemblage experiment

B) Removal experiment

C) Survival at day 28

0.045). There was no difference between the 15 species and 30 species treatments (Tukey's test, $P > 0.05$).

In the removal experiment, the cell densities of *E. coli* also progressively declined throughout the experiment (Fig. 2B). The decline was influenced by time, species richness, and the interaction between these two factors (Table 1). Differences among treatments were observed as from day 5, and the differences were exacerbated throughout the experiment (Fig. 2B). While there was no statistical difference in *E. coli* survival between the 10^{-1} and 10^{-3} treatments (Tukey's test, $P > 0.05$), these two treatments incited significantly reduced *E. coli* abundances when compared to the 10^{-6} and sterile soil treatments (Tukey's test, $P < 0.05$). Moreover, invader survival in the sterile soil was significantly higher than in all other treatments throughout the experiment (Tukey's test, $P < 0.05$). At day 75, *E. coli* had already gone below the detection limit (500 CFU/g soil) in the 10^{-1} and 10^{-3} treatments. Yet, it was still detectable in the 10^{-6} treatment, and it maintained a high abundance in the sterile soil.

E. coli abundance values for the assemblage experiment were interpolated at day 28 in order to compare them to abundance values observed in the removal experiment at the same day, which represents a sufficient lapse of time to portray invader survival dynamics. The relationship was strong and negative ($R^2 = 0.92$, $P < 0.0001$), indicating that the species richness gradient found throughout the two experiments strongly and consistently affected *E. coli*'s survival (Fig. 2C).

Community niche, remaining niche available to the invader, and invasion success

The community niche calculated from both experiments was strongly and positively related to species richness (Fig. 3), indicating the importance of resource complementarity in communities of higher richness across both experiments. In addition, in the assemblage and removal experiments, strong negative correlations were observed between *E. coli*'s abundance and the community niche of the resident community at each sampling day (Fig. 4). Also the invader abundance displayed positive and significant relationships with the remaining niche available to the invader in both experiments for all sampling days (Fig. 5). The two metrics employed to assess the invader's fate, community niche and the invader's remaining niche availability,

FIG. 2. Progressive temporal decline in invader abundance across a gradient of species richness in communities established by (A) assembling consortia of bacterial strains or (B) decreasing the diversity of a natural soil via a removal approach. Numbers in parentheses on the key are the number of species (mean ± SE) in that dilution. Dashed lines in panel (B) indicate that *Escherichia coli* had fallen below the detection limit of 500 cells/g soil upon quantification at day

75. (C) Actual survival values of the invader at day 28 (removal experiment; filled squares) and interpolated values of invader survival at day 28 (assemblage experiment; open squares) showcase a consistent relationship between invader survival and community diversity among the two experiments. Vertical bars represent mean ± SE for *E. coli* abundance. Horizontal bars represent mean ± SE for observed number of operational taxonomic units (OTUs) in the removal experiment. Survival was measured as colony-forming units (CFU) per gram soil.

TABLE 1. Two-way ANOVA testing for effects of richness, time, and their interaction on invader abundance in each experiment.

Source of variation	df	MS	F	P
Assemblage experiment				
Richness	3	57.4	129.5	<0.0001
Time	3	172.7	389.5	<0.0001
Richness × time	9	6.5	14.7	<0.0001
Error	190	0.4		
Removal experiment				
Richness	3	16.6	94.1	<0.0001
Time	3	10.0	57.1	<0.0001
Richness × time	9	0.7	4.2	0.001
Error	32	0.2		

Note: MS is mean square.

explained a larger portion of the variation of invader survival in the removal than assemblage experiment.

Resource pulse and E. coli survival

The resource pulse (addition of D-galactose at day 105 to selected microcosms of the assemblage experiment) resulted in an increase of *E. coli* abundance in all treatments, but more particularly in the highest richness treatment (30 species), which had nearly gone undetected (Fig. 6A). Fifteen days after the addition of carbon, *E. coli* abundance was highest in the sterile soil, followed by the 5, 15, and 30 species treatments, respectively. From days 107 to 115, the abundance of *E. coli* increased 69% in the 5-species and 93% in the 15-species treatment. Strikingly, an even stronger response was seen in the 30-species treatment, as *E. coli* regrew over seven times its abundance. In order to evaluate how the community's use of D-galactose would affect the regrowth of the invader, we examined the bacterial resource-use profiles of individual strains to extract the maximum value of D-galactose use among each community and used it to predict invader abundance after the resource pulse (Fig. 6B). The community's maximum use of D-galactose could significantly predict invader abundance at day 107, but it was not significant thereafter. Furthermore, we examined what effect species richness had on invader regrowth across days 107, 110, and 115 (Fig. 6C). The variance explaining invader regrowth declined as of day 110, and the slope of the invader abundance–richness relationship became less and less negative with time after the resource pulse (Fig. 6C).

DISCUSSION

Several previous studies on biological invasions, mostly in plants, highlight the importance of resource availability and associated competition in determining the fate of invasive species (Davis et al. 2000, Tilman 2004, Romanuk and Kolasa 2005, Li and Stevens 2012). Yet, the importance of resource competition is still under debate because other authors have concluded that diversity by itself was the key factor in resistance to invasion, regardless of resource availability (Maron and Marler 2007, Roscher et al. 2009, Liu et al. 2012). Here, we showed that, for soil microbial communities invaded by *E. coli*, increased levels of competition were driven by increased levels of species richness and reduced the remaining niche available to the invader, causing its progressive elimination from the community. This underlying relationship between richness and invasion could be alleviated by the application of a resource pulse.

Specifically, our results show that invader survival decreases with increasing species richness of the resident microbial community ($R^2 = 0.92$), which is in line with reports from other microbial invasion experiments (van Elsas et al. 2007, 2012). We demonstrated that this relationship is consistent over a large range of species richness values (from 0 to 438 species), independent of
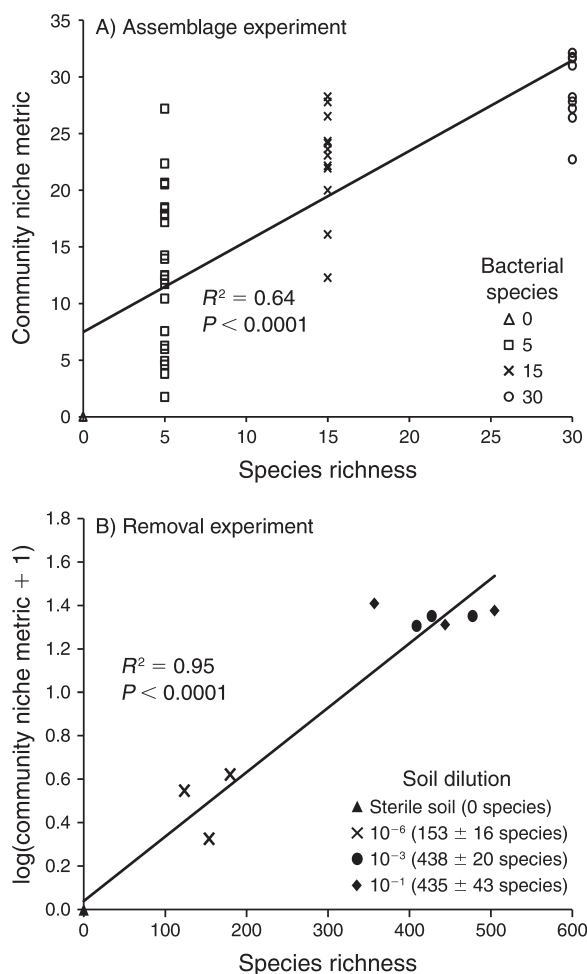

FIG. 3. In both the (A) assemblage and (B) removal experiments, increased species richness correspond to increased performance of communities, as measured by the community niche metric (see *Methods: Quantification of soil community niche and remaining niche available to the invader*). Numbers in parentheses on the key are the number of species (mean ± SE) in that dilution.

A) Assemblage experiment
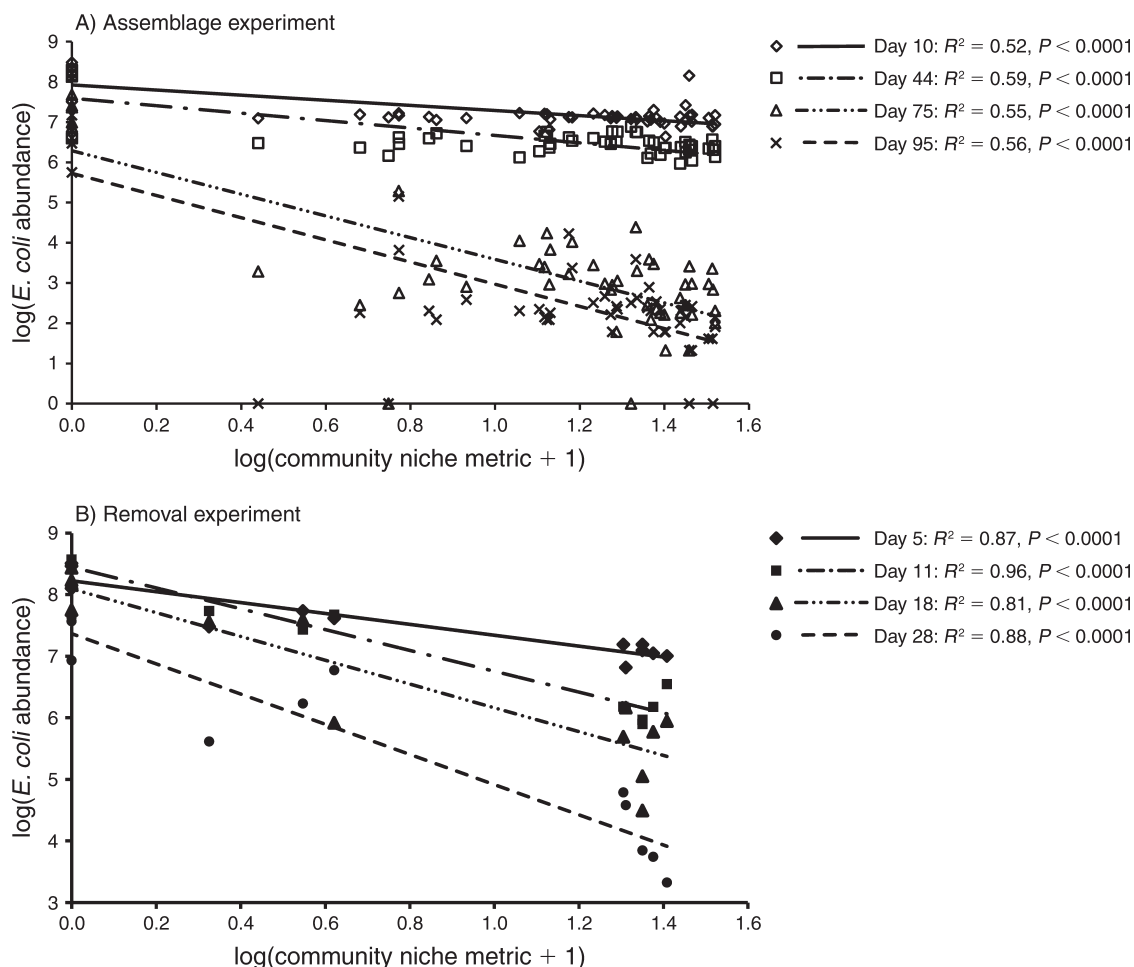

B) Removal experiment

Fig. 4. Invader success is correlated with (A) the community niche of assembled soil communities and (B) the community niche of soil communities obtained by the removal approach. For the removal experiment, correlations from day 75 were omitted, since *E. coli* was already below the detection limit in some treatments. Abundance was measured as CFU/g soil.

the experimental approach used (i.e., removal or assemblage experiment). Moreover, we provide a mechanistic interpretation of this diversity–invasion relationship by applying metrics designed to accurately predict the functioning of bacterial communities, which take into account the different abilities of species within a community to use an array of substrates. The positive relationship observed between community niche and species richness for each experiment is indicative of resource complementarity among the more diverse communities (Salles et al. 2009). Increased levels of complementarity across both experiments led to better use of carbon sources and ultimately increased competition toward the invader in more diverse communities. This was apparent given the decrease in the remaining niche available to the invader, which accurately predicted the invader's abundance across both experiments at each sampling day. In line with our hypothesis in Fig. 1A, our results thus strongly support that the resource-use capacity of communities, which in our case and

other reported cases are often positively linked to species richness (Bell et al. 2005, Langenheder et al. 2010), can restrict biological invasions. While we acknowledge that we used a proxy for what is likely to happen in natural soil communities, the strength of the observed relationships and the consistency of the results obtained from the two independent experiments indicate that our results are robust. Our experiments differ from other invasion experiments that have elucidated resource use as a driver of invasion resistance (Stachowicz et al. 1999, Dukes 2001, Hector et al. 2001, Fargione and Tilman 2005) in that, instead of quantifying the amount of resources left over in the system after a defined period of invasion, we directly quantified the resource-use capacities of the experimental communities and the remaining niche available to the invader. This arguably provides a more direct link between the community and its likely resource utilization pattern and demonstrates that using metrics based on microbial functional traits (Krause et al. 2014) can promote more predictive microbial
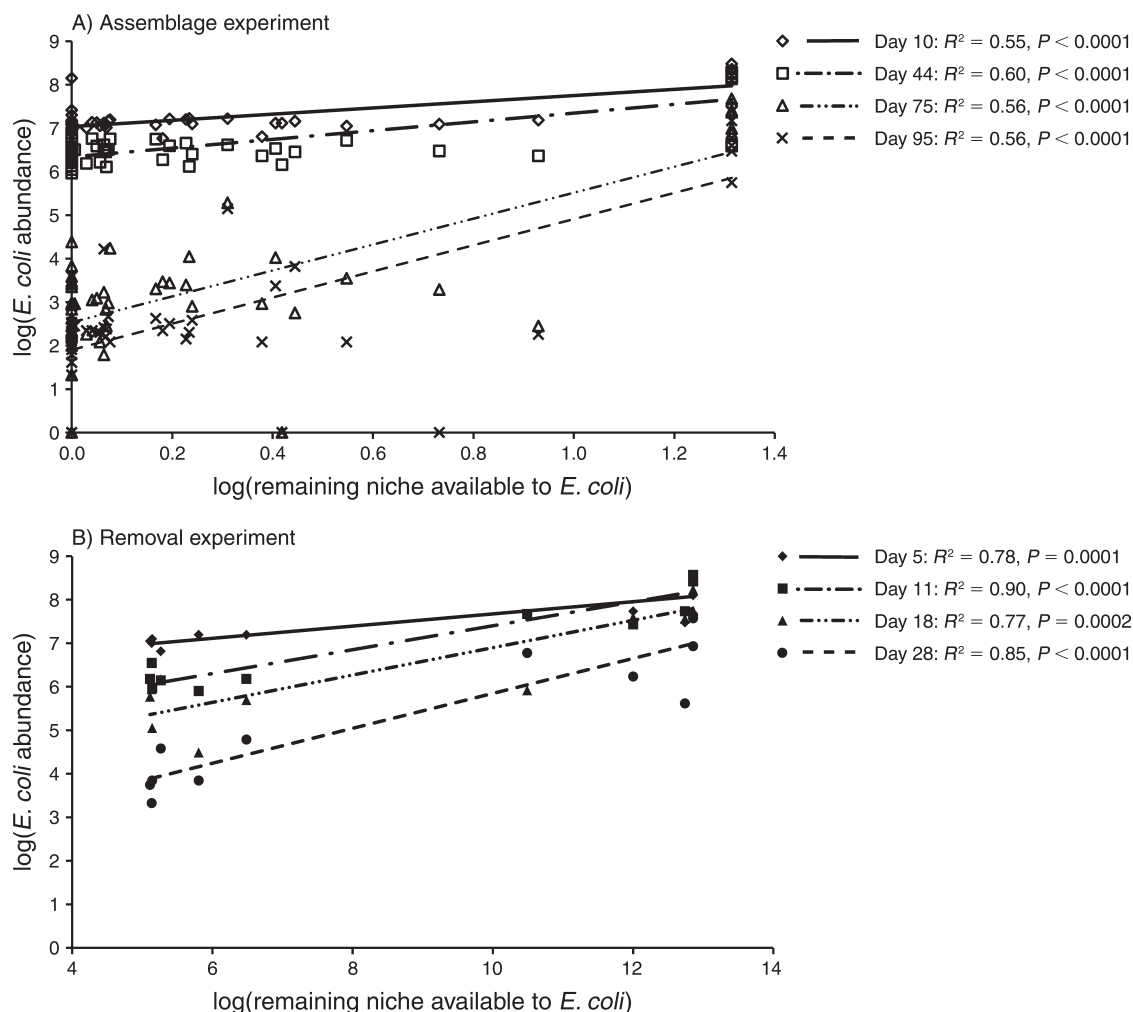
Fig. 5. The remaining niche available to *E. coli*, calculated by examining *E. coli*'s advantage over the resident community on the array of 71 carbon sources, explains its persistence in both the (A) assemblage and (B) removal experiments at each sampling date. Abundance was measured as CFU/g soil.

ecological studies. Furthermore, although the invader did not increase in abundance in any of the diversity treatments, it did manage to survive for an extended period of time (i.e., at least 28 days) across all treatments in both experiments. This lends support to the theory that biotic resistance (i.e., competition or diversity-related mechanisms) controls the spread and growth of the invader after it has become established rather than upon initial introduction into the community (Levine et al. 2004).

In order to confirm the importance of resource availability, we applied a resource pulse to a portion of invaded communities in the assemblage experiment. The observed effect of the addition of D-galactose supports the claim that lack of available carbon was indeed restricting *E. coli* survival. Interestingly, after the addition of D-galactose, *E. coli*'s abundance was able to rebound to near inoculation levels. This adds support to the fluctuating resource hypothesis that states that an

influx of resources increases the invasibility of a community (Davis et al. 2000). Since established invaders often struggle to spread and grow (Levine et al. 2004), an influx of resources provides an opportunity to expand populations through the alleviation of competition. This likely allows established organisms to grow and spread. Our results also support our hypothesis presented in Fig. 1C, which states that resource pulses can alleviate competition and reverse the survival trajectory of an invader nearly eliminated from the soil community. In contrast, Liu et al. (2012) showed that even if resources inputs before invader introduction enhanced the invasion success, the structure and diversity of the community was more important in determining the invader's success. This experiment, however, did not examine resource competition between the resident community and invader, measuring survival only in treatments of varying diversity where different amounts of the resource were added.
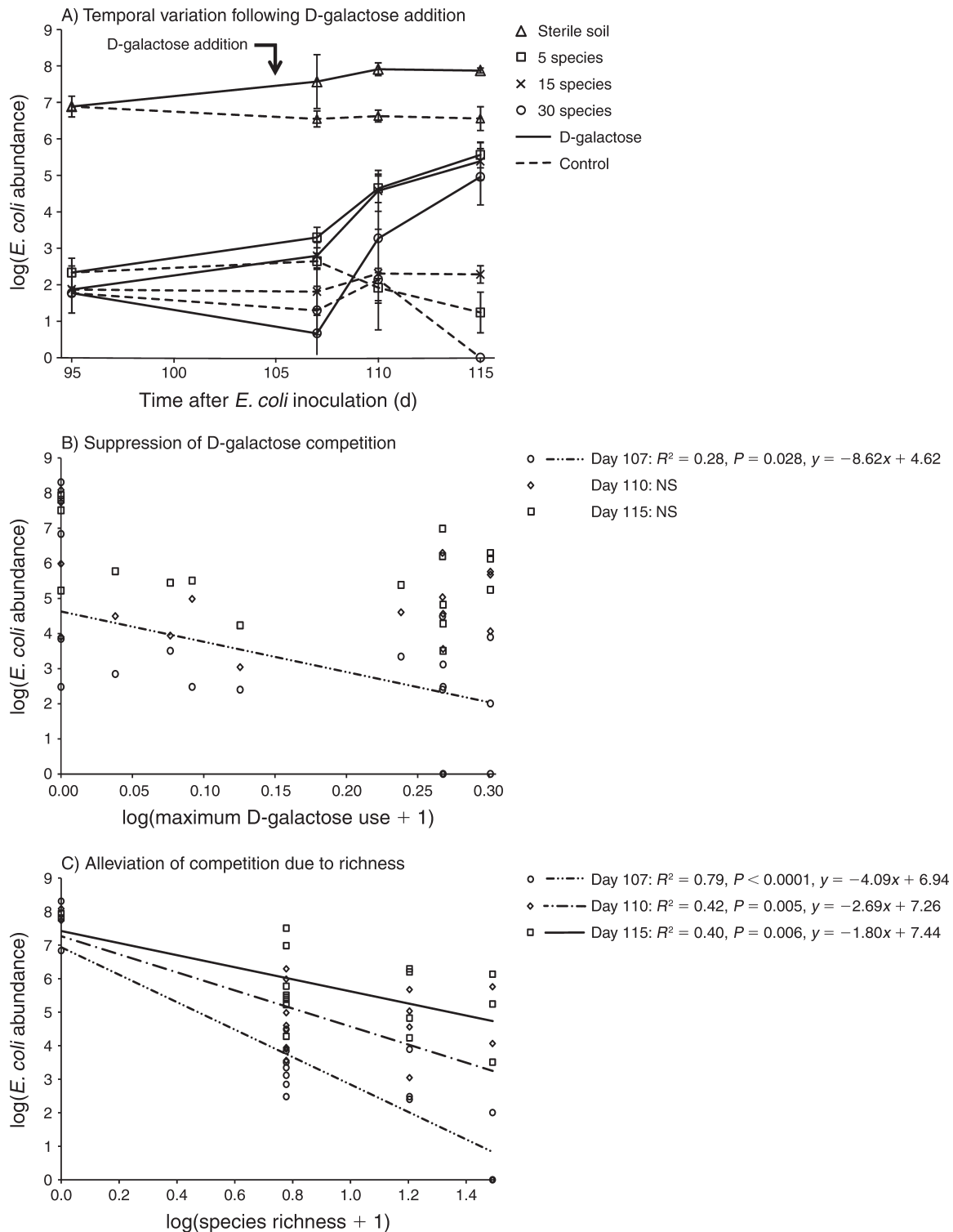
FIG. 6.   Effect of a resource pulse on invasion and diversity–invasion relationships. (A) Temporal variation in the invader abundance following an addition of D-galactose 105 days after invader inoculation. A portion of the replicates from the assemblage experiment received D-galactose (solid lines) or sterile water (control treatment; dashed lines). Bars represent standard errors. Panels (B) and (C) present the relationship between the invader abundance and the community's maximum use of D-galactose and the community's species richness, respectively, for the three sampling dates following the resource pulse. The community's maximum use of D-galactose was determined by using individual bacterial strain profiles to identify the maximum value of D-galactose use in each assembled community (measured as omnilog units). Abundance was measured as CFU/g soil.

The effect of the resource pulse on the diversity–invasibility relationship is remarkable in our study given the timing of the resource pulse. Indeed, Li and Stevens (2012) found that resource pulses were most effective when added coincidentally with the invader rather than before or after invasion. In our study, the resource pulse occurred when the invader had already significantly decreased in abundance. Nevertheless, this allowed a strong rebound of the invader cell density. Li and Stevens (2012) demonstrated that resource pulses will differentially affect different invaders, and this was probably due to the different physiological requirements and ecological strategies of the different species studied. For instance, opportunistic species with fast growth rates may benefit from any resource pulse, whether it occurs before, after, or upon their invasion. Alternatively, resourceful invaders with slow growth rates may benefit only from resource pulses coincident with invasion that allow them to form established populations and conserve resources until the next pulse. Understanding how different invaders with different competitive abilities respond to resources pulses across a diversity gradient are thus needed.

Other mechanisms, which are not necessarily related to resource use, may also have been at play throughout both experiments. Likely candidates are antagonism, predation, and parasitism. Evidence has shown that antibiotic-producing bacteria can grow from rare to dominant species in a community due to the elimination of competitive species and subsequent acquisition of resources (Chao and Levin 1981). In the context of diversity, antagonistic effects can vary depending on the genotypic richness of the community, as model microbial communities have been shown to reduce invader success at low levels of diversity (up to four genotypes) while this effect was alleviated, perhaps due to self-poising of resident community members, in higher diverse communities (Jousset et al. 2011). Predation from higher organisms like protozoa can also decrease invading bacterial populations by grazing on bacterial invaders (Postma and van Veen 1990). Moreover, although less likely to occur due to the high specificity required between phage and host, soil bacterial invaders may also be combated by virulent phages that lyse host cells upon infection (Marsh and Wellington 1994). Further investigations into these three factors, in conjunction with resource use, resource availability, and species richness are needed to understand the full gamut of invasion resistance in natural microbial communities and may lead to a more complete view of soil microbial invasions. Still, the metrics of community niche and remaining niche available to the invader always explained more than one-half of the variance (up to 96%) of an invader abundance that was in constant decline, and the resource pulse induced a resurgence of the invader, in particular when its abundance had become very low in more diverse communities. In accordance with our initial hypotheses, this clearly shows that (1) resource competition suppresses invasion in the microbial systems studied, (2) community diversity increases resource competition and decreases invasion through niche preemption, and (3) resource pulses that cannot be fully used even by diverse communities are favorable to invasion.

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SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A–F are available online: http://dx.doi.org/10.1890/14-1001.1.sm