INVASIONS AND DIVERSITY: MECHANISMS, MICROBES, AND MULTIPLE SCALES

By

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ABSTRACT OF THE DISSERTATION

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As anthropogenic influences on biological communities increase around the world, understanding how community level processes interact to affect ecosystem functioning has become a critical area of current ecological research. Biological invasions are one of the major drivers in biodiversity losses on local scales. Where exotic species do not perform comparably to the native species that they replace, they can be expected to have important consequences for ecosystem functioning. The question of which traits contribute to improved invasion potential is still a major focus of the field of invasion biology. In the first chapter of my dissertation, I examined how exotic and native shrubby plant species found in New Jersey interact below ground to influence competitive interactions in a greenhouse experiment. I found that exotic plants did not uniformly outperform native plants for a contested patch of soil nitrogen, but instead the native *Rubus allegheniensis* and exotic *R. phoenicolasius* both grew to significantly larger size than other competing plants and reduced competitor growth by greater than 50 percent. These results indicate that while root competition appears to be important for these shrub species, exotic species do not routinely outcompete native ones, suggesting that root competition among shrubs may not be an important trait governing invasion success.

To fully understand the consequences of biological invasions, we must also understand how biological invasions affect invaded communities over evolutionary time. The remaining chapters of my dissertation used laboratory microcosms to experimentally study how evolution among resident and invading species altered the consequences of biological invasions.

In Chapter 2, I determined that evolution resulted in increased performance for one invader, *Euplotes daidaleos*, in an assemblage of protists and rotifers, while in a second assemblage, evolution resulted in the appearance of increased biotic resistance of resident species against an invader, *Paramecium bursaria*. Changes in performance of both resident and invading species also resulted in significant differences in community composition in both assemblages. In one assemblage, communities with evolved invaders were most similar in composition, while in the other assemblage, communities with evolved residents were most similar.

In the third chapter of my dissertation, I examined how evolution altered the temporal variability in species abundances. I found that for *E. daidaleos* the pattern of temporal variability in abundance most closely matched predictions of evolution decreasing the attack rate of predators on *E. daidaleos,* implying that this invader may have evolved increased defense against predators that were resident species of the assemblage into which it invaded. Several resident species in this community experienced decreased abundances concurrent with increased temporal variability in abundance suggesting one mechanism by which evolution may predispose those populations to extinction, while decreased temporal variability in invader abundance could facilitate invader persistence. These effects on community dynamics may provide one mechanism to explain how evolution can exacerbate invasions in some communities and ameliorate invasions in others.

In Chapter 4, I determined how ongoing evolution in invaded communities affected biomass production, a measure of ecosystem functioning. I determined that biomass production tended to mirror species performance in abundance, but evolution ultimately reduced ecosystem functioning below that observed in the uninvaded state for both communities. These effects highlight the risk inherent in assuming that the long-term consequences of invasions can be adequately predicted by the effects observed following initial invasions by naïve species into novel communities.

Collectively, these chapters, though different in theoretical motivation and empirical approach, demonstrate the complex nature of biological invasions, highlighting the need to consider the consequences of biological invasions at multiple ecological scales and time scales. To truly understand the consequences of invasions we must remember that the contemporary interactions that we observe today represent a snapshot in time, and consequently may not adequately predict future interactions. This dissertation particularly illustrates the point that each new biological invasion is a process and urges caution in our interpretation of the outcome of that process.

DEDICATION

I dedicate this dissertation with love to my parents, John and Carol Faillace, who have always encouraged and supported me in all of my endeavors.

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INTRODUCTION

Ecosystem functioning, which is sometimes measured by how productive communities are and how well they capture and consume resources, is dramatically affected by community composition and biodiversity (Loreau 2010). The importance of biodiversity in determining ecosystem functioning is of great interest to the scientific community, in part due to biotic homogenization and the increasing number of local extinctions that affect biodiversity (Schmid et al. 2009). Biological invasions have contributed to the loss of biodiversity at local, regional, and global scales (Russell & Blackburn 2017). The often superior abilities of exotic species to fill niches in biological communities enable them to achieve high abundances in their non-native ranges, often resulting in significant ecological damage (Lockwood et al. 2007). The unusually high local abundances of invasive species may additionally indicate that they can have a stronger net effect on ecosystem functioning than do native species. This is one driving force behind the question of which traits contribute to improved invasion potential, a major focus of the field of invasion biology.

Although in some situations the presence of exotic species simply increases biodiversity (Meiners 2007), invasive exotics may present considerable challenges to restoration ecologists and land managers concerned with preserving native biodiversity and ecosystem functioning. Scientists have yet to fully explore whether exotic species affect functioning similarly to native species when their presence in invaded communities increases or decreases biodiversity. Invasive species also result in significant economic losses (in the billions of U.S. dollars per year) due direct damage, as well as the costs of control measures, making them of both economic and ecological importance across the world (Pimentel et al. 2005). New invasive species are identified around the globe at an increasing rate, implying that their potential to influence invaded communities, positively or negatively, will also continue increase.

Some of the factors influencing a species' invasion potential include the degree of climate matching between its native and introduced ranges, the number and location of introductions, the number of individuals introduced, genetic diversity of the population in the non-native range, and the presence or absence of mutualists and natural enemies (Lockwood et al. 2007). For example, escape from natural enemies is predicted to play an important role in the invasion success of a diverse group of invaders, and successful biological control efforts point to the importance of this factor for some invaders (Lockwood *et al.* 2007; Charudattan 2010; Morin 2011). Tests of biotic resistance (*i.e.*, that species interactions can effectively resist colonization or expansion by invaders), have produced mixed results (Maron & Vila 2001; Parker & Hay 2005; Hoffmann & Saul 2010), further highlighting the idiosyncratic nature of biological invasions. The susceptibility of biological communities to invasion can be affected by diversity or species richness, levels and frequency of disturbance, and species interactions such as competition, predation, and mutualistic interactions (Lockwood et al. 2007). Post-invasion evolutionary changes that enhance the performance and fitness of invading and native species alike may also be important, governing invader success and community invasibility especially at longer time scales. Incomplete records of invasion history and early community dynamics, however, make it difficult to study species interactions that transcend ecological time into evolutionary time. Current understanding of the consequences of biological invasions is therefore primarily limited to relatively short time scales and conjecture (Strauss *et al.* 2006b; Dlugosch & Parker 2008).

Competitive interactions between native and invasive species have garnered a great deal of interest due to their importance in both the invasion process and the community level effects following invasions (Knevel *et al.* 2004; deRivera *et al.* 2005; Eppinga *et al.* 2006). Research examining competitive interactions between native and invasive species has improved understanding of how invasive species might affect native species and what sort of interactions between native and invasive species may be important (Richardson & Pysek 2006), but has not addressed how these interactions are likely to play out in diverse biological communities. Studies addressing the interactions between multiple native and invasive species interacting together are, however, essential to understand the community and ecosystem level impacts of biological invasions (Alvarez & Cushman 2002).

In terrestrial communities, belowground interactions among invasive plants and native plants may have important consequences for invasion success as well as in determining plant community species richness because root biology and behavior can be an important determinant in plant growth and overall competitive ability (Fargione & Tilman 2006). Roots are responsible for the essential function of nutrient and water uptake in most vascular plants, as well as for physical stabilization (Fitter 1991). Their role in soil nutrient and water acquisition means that competition mediated by roots often plays an important role in determining the competitive outcomes between plants when they share rooting zones (Wilson 1988; Coomes & Grubb 2000).

The relative importance of root versus shoot competition in determining outcomes of competitive interactions has garnered much interest, with generally mixed results (Dehlin *et al.* 2008; Chauhan & Johnson 2010). Results differ in terms of which type of competition is more likely to have a greater effect on individual plants, and it is likely that the particular abiotic conditions examined greatly influence the relative intensity of shoot and root competition (Chen et al. 2008). Research in a variety of systems suggests that root competition intensity generally increases with increasing nutrient and water limitation (Goldberg *et al.* 1999; but see also Belcher *et al.* 1995 for the opposite finding). Additionally, the distribution of resources may also affect the intensity of competition. Research indicates that root competitive intensity can be greater when soil resources are distributed heterogeneously (O'Brien *et al.* 2005; Tuma *et al.* 2009). Competition for patchy soil resources can also have an impact at the community level by decreasing plant diversity (Rajaniemi 2011).

Invasion biologists have begun to turn their attention belowground to examine the root interactions among invasive and native plants (Lopez-Zamora *et al.* 2004; Schenk 2006). Root competition among native and invasive plants can be quite intense (Kueffer et al. 2007), and some invasive species show higher resource use efficiency and greater biomass allocation to roots than do the native competitors (Drenovsky et al. 2008). Root physiological plasticity may explain how some invasive forbs capture more nitrogen in heterogeneous soils compared to native species, potentially making them superior competitors for soil resources (James et al. 2009). Some invasive plants demonstrate allelopathic capabilities that not only impact plants in direct competition for resources, but can also suppress native seedling germination (Dorning & Cipollini 2006; Ens *et al.* 2009; Murrell *et al.* 2011). It is clear that root competition can play a role in biological invasions, but it has yet to be determined how frequently it does so.

One of the greatest effects of biological invasions is altering community composition in invaded sites. Community composition and complexity can have strong interactions with ecosystem functioning, making the changes to community composition caused by biological invasions potentially of great importance to continued ecosystem functioning (Schmid 2002). Results of current studies provide support for the importance of biodiversity in maintaining both high levels of ecosystem functioning and low variability in functional properties (Cardinale *et al.* 2012; Naeem *et al.* 2012; Tilman *et al.* 2014). Biodiversity may influence ecosystem functioning through several mechanisms, including niche complementarity, sampling effects, and the insurance hypothesis (Morin 2011). Regardless of the specific mechanism driving the relationship between biodiversity and ecosystem functioning, biological invasions that decrease species richness may be expected to have significant effects on ecosystem functioning.

In addition to understanding the causes and consequences of recent biological invasions, it is equally important to understand the longer term consequences of invasion events. Hypotheses such as enemy escape and biotic resistance (Elton 1958) are often invoked to explain invasion potential and community invasibility, but our understanding of how the importance of factors such as these will change over evolutionary time scales is very limited. Invasive species often experience novel selective pressures in their introduced range, making them ideal for the study of how selection pressure affects the evolution of traits that affect performance; research of this sort is still in its early stages (Barney et al. 2009), however, and much remains to be done. Nonetheless, questions concerning evolutionary changes following invasion events are of the most fundamental and essential importance. Evolution has the capacity to change the course of biological invasions by influencing invader success and native species' responses and modifying the impacts of biological invasions (Carroll 2011) through effects on interspecific interactions (*e.g.*, see Palkovacs *et al.* 2009; Schoener 2011).

Strong evidence for evolution requires measuring traits before and after invaders and residents have interacted for some time (*e.g.*, Stuart *et al.* 2014), so research aiming to definitively assess how evolution affects long-term invasion dynamics should measure the traits of both resident and invading species early and later in the course of invasions (*e.g.*, see Kolbe *et al.* 2012). Because evolution is a dynamic process, we might also expect that initial evolutionary responses may change over time. Unfortunately, traits of resident and invading species are often characterized early in the course of invasions, before much evolution can occur, or much later, after evolution may have occurred, but when initial trait states are no longer available for comparison. Inferences about ongoing evolution are frequently further complicated by an incomplete knowledge of the history of introductions and spread for invading exotic species (Le Roux & Wieczorek 2009; Kolbe *et al.* 2012). These limitations can be addressed by experiments using laboratory organisms with short generation times. The opportunity for potential evolution can be altered by varying the duration of post-invasion interactions (Warren et al. 2006), while measuring evolutionary outcomes over experimentally tractable time spans. It is impossible for us to fully understand the long-term consequences of invasion if we do not understand how invasive species affect native communities over both ecological and evolutionary time scales.

With this dissertation, I explore the short-term ecological phenomena of interactions among native and invasive species, using plants in a nearby habitat, and the long-term consequences of biological invasions using microcosms where generation times are short enough to allow for studying evolution in a tractable fashion.

In Chapter 1, I explored how root competition among native and exotic shrubs of New Jersey for a contested patch of soil nitrogen affects plant growth and overall biomass. In particular, I determined whether two focal exotic shrub species outcompeted two focal native shrub species. The remaining chapters of my dissertation used laboratory microcosms of aquatic microorganisms to study how evolution among resident and invading species altered the long-term consequences of biological invasions in experimental communities.

In Chapter 2, I determined whether evolutionary experience of invading and resident species altered species performance and community composition.

In the third chapter, simple two-species models of Lotka-Volterra competition and predator-prey interactions generated predictions for how evolution might alter the temporal variability in species abundances, and I empirically tested the effects of evolution on temporal variability in consumer species abundances.

In the fourth chapter of my dissertation I examined how evolutionary experience ultimately altered ecosystem functioning in invaded communities by determining how biomass changed as a result of evolutionary experience of invading and resident species in the same experimental laboratory communities.

As a whole, this dissertation provides new information about the role of belowground competition in determining the success of invading shrubs as well as providing much needed insight into the long-term community- and ecosystem-level consequences of biological invasions arising out of evolutionary change to invaders and residents alike. Here I have provided a first step forward in assessing the effects of evolution resulting from biological invasions on community and ecosystem properties, but the study of eco-evolutionary dynamics of biological invasions remains a critical area of research as we seek to understand the importance of invasive species more fully.

CHAPTER 1

Beneath it all: size, not origin predicts belowground competitive ability in exotic and native shrubs

Abstract

Traits associated with root morphology and nutrient uptake rate contribute to the competitive ability of invasive species by determining access to soil nutrients and ability to extract those resources. Here, we tested the hypotheses that 1) exotic woody shrubs would be superior belowground competitors for a limiting resource (i.e., nitrogen) in heterogeneous soil resulting from key aspects of root architecture and 2) larger plants would be superior root competitors. We tested this hypothesis using two native shrubs, *Rubus allegheniensis* and *Viburnum dentatum*, and two invasive exotic shrubs, *R. phoenicolasius* and *Berberis thunbergii*; all four of which can become abundant in plant communities in the eastern U.S. We grew replicate plants from each species with interspecific competitors, with intraspecific competitors and individually in a randomized layout in a greenhouse. Each experimental container had a central patch amended with ^{15}N-labeled litter. We measured above- and belowground growth, root morphology and N uptake to assess the effects of intra- and interspecific competition on plant growth and N uptake. We did not detect any differences in the competitive ability or root traits of exotic versus native species, rather size was the key trait that predicted competitive effects. Both *Rubus* species were stronger competitors and typically larger plants than *B. thunbergii* and *V. dentatum*. Both *Rubus* species exerted measurable competitive effects on other species, resulting in decreased aboveground size of competitors by 50% or more relative to control plants, but did not routinely decrease ^{15}N uptake or root biomass of competitors. When competing with *Rubus*, leaf C:N ratios of all species except *Rubus phoenicolasius* were greater than when grown alone, suggesting that large *Rubus* plants did decrease the total nitrogen available to competitors. Belowground competitive ability in shrubs may be associated with plant size and growth rate rather than plant origin. Plant species that exhibit plastic growth phenology, such as those in the genus *Rubus*, may gain a competitive advantage during years with warmer autumn months by extending their growing seasons, facilitating their invasion and establishment in new habitats.

Key words: belowground competition; exotic plants; nitrogen; roots; woody shrubs

Introduction

The role of roots among native and exotic plant species is rarely addressed in the search for traits that contribute to invasion success, but could help explain invasiveness and post-invasion impacts (Fargione & Tilman 2006). Root traits and belowground competition may be especially important in forested systems where many of the most damaging invasive species are woody shrubs (Webster et al. 2006). Interspecific differences in root morphology and nutrient uptake rate may contribute to the competitive ability of exotic shrub species by determining both access to soil nutrients and an ability to extract those resources (Wilson 1988; Coomes & Grubb 2000).

Root biomass can determine a plant's nitrogen acquisition in competition (Gaudet & Keddy 1988; Lamb *et al.* 2012), but the effect of nutrient and water limitation on the intensity of root competition is debated (Wilson 1988; Belcher *et al.* 1995; Schenk 2006). Some studies suggest that plant species that possess a superior ability to acquire nutrients benefit disproportionately in high resource environments (Gioria & Osborne 2014), and both root and shoot competition are strongest when soil nutrients are abundant and plants are actively growing (Grime 1977; Li *et al.* 2010; Li *et al.* 2015). A recent systematic meta-analysis, however, has reported the opposite result: that root competition intensity is generally higher in low resource environments (Kiaer et al. 2013).

The persistence of exotic shrubs in the forest understory makes them a dominant component of invaded northeastern North American forests, at times forming dense, nearly monospecific stands that competitively exclude native plant species (Dorning & Cipollini 2006). Spread by exotic species may be further encouraged through the ongoing and widespread nutrient enrichment in temperate forests by favoring species that are fast-growing or superior belowground competitors and are able to persist in forest understories (Gilliam 2006; Perry *et al.* 2010). Although the contribution of root traits to plant competitive ability may be particularly important for exotic woody shrubs because their perennial root systems occupy soil continuously for long periods of time, the majority of studies examining the effects of root traits on belowground competitive ability have focused on herbaceous species (Roumet *et al.* 2006; Dehlin *et al.* 2008). Comparable research on woody shrubs remains sparse, especially for exotic shrubs.

This study addressed whether understory shrub species from forests of northeastern North America exhibit consumptive competition for nitrogen in heterogeneous soils and whether interspecific differences in root morphological and physiological traits are associated with competitive ability. We chose four shrub species based on their high prevalence in deciduous forest understories of northeastern North America. Two are native to the region, *Rubus allegheniensis* Porter (Allegheny blackberry) and *Viburnum dentatum* L. (arrowwood viburnum). The other two species, *Berberis thunbergii* DC (Japanese barberry) and *Rubus phoenicolasius* Maxim (wineberry), are exotic species from genera known to have negative effects on native plants in invaded communities (Ehrenfeld 1999; Silander & Klepeis 1999; McDowell 2002; Lambrecht-McDowell & Radosevich 2005; Xu *et al.* 2007; Gorchov *et al.* 2011). We hypothesized that 1) exotic shrubs would outperform native shrubs when grown with an interspecific root competitor and 2) larger plants would be superior root competitors.

Materials and Methods

Experimental Setup

Plant propagation took place at Pinelands Nursery (Columbus, NJ) using seeds collected from local populations of the respective species and cold-stratified before germination. The competition experiment took place in a greenhouse run in two consecutive temporal blocks because we were unable to fit all experimental containers in one greenhouse room at one time. We planted seedlings of roughly equivalent size (under 15 cm in total stem length) and age in containers using a full-factorial design. Factors included Species (4 levels) and Competitor (5 levels; none or each species), yielding 14 non-redundant treatment combinations. Containers were rectangular boxes of white corrugated plastic (46 × 20 × 25 cm) filled with field soil amended with sand yielding a composition that was 19% gravel, 48% coarse sand, 19% fine sand, 13% silt and clay by mass. Field soil used in block two had roughly 8 times the total N content of field soil used in the first block (219.3 vs. 27.4 μg) due to variability in supply. To evaluate the importance of contested patches to competitive outcomes, each container included a vertically-oriented band of ^{15}N enriched litter combined with the same soil mixture (0.75% *Lolium multiflorum* by mass, enriched to 4.2 atm %) placed across the center of the container. White corrugated plastic dividers aboveground between plants ensured that competition occurred without the influence of shoots (von Wettberg and Weiner (2003). Control containers contained each species grown singly under identical conditions.

Containers were randomly placed on one of four greenhouse benches at the start of either of two temporal blocks (26 weeks each). We initially established 11 to 12 replicates of each treatment combination, targeting 10 replicates after early plant loss. Final sample sizes ranged from 8-11 replicates of each treatment combination. We watered the soil in each container to field capacity 1-3 times per week, with watering dependent on plant size and abiotic conditions in the greenhouse. Greenhouse shades were drawn when photosynthetic photon flux density exceeded 200 μmol m^{-2} s^{-1} and supplemental lighting was used to maintain a 16:8 hour light: dark photoperiod in both temporal blocks. Temperature set points ranged from 20.5° C to 23.9° C during the day and from 12.8° C to 15.6° C at night. Plants in the first block grew from August 2011 until February 2012, while plants in the second block grew from May until October 2012. Each block was terminated when we determined that larger plants were becoming pot-bound.

After 26 weeks, we harvested all aboveground plant material. We measured total stem length, counted the number of leaves, measured total leaf area using a LI-COR 3100C Area Meter (LI-COR Biosciences, Lincoln, NE) and then measured oven-dry biomass of all shoots. We finely ground and homogenized leaf tissue for ^{15}N, ^{13}C, and C:N analysis using continuous flow isotope ratio mass spectrometry (UC Davis Stable Isotope Facility, Davis, CA).Carbon-13 was opportunistically analyzed to determine if the identity of a root competitor affected water resource use efficiency (Warren et al. 2001). Samples sizes for tissue chemistry ranged from 6-10 samples for each treatment combination. We then washed all soil away from root systems, measured the lengths of the longest lateral roots from each plant and scanned this root (together with its daughter roots) on a flatbed scanner (600 dpi). Images were analyzed for total fine root length using WinRhizo 2007d (Regent Instruments, Quebec, Canada) before measuring oven-dry biomass of all root material.

Statistical analyses

We used multivariate analysis of variance (MANOVA) to analyze effects of species and competition on leaf number, total stem length, leaf area, aboveground biomass, belowground biomass, longest root length and total fine root length after $\log_{10}$-transforming responses to help satisfy assumptions of residual normality. Because measures of intraspecific competitors within containers were not independent, we averaged responses of both intraspecific competitors grown within a pot. This method retained the container as our experimental unit and ensured that our samples were independent. We first compared above and belowground growth characteristics among our four focal species using plants grown without competitors. We then examined growth characteristics within each species for effects of intra- or interspecific competitors using MANOVA with mixed effects. The primary fixed effect was competitor identity; we included random effects to account for variability between temporal blocks and for competitor × block interaction effects. Additional MANOVAs for each species examined the effects of root competition on leaf ^{15}N enrichment, ^{13}C enrichment, and leaf carbon to nitrogen (C:N) ratio. Treatment means were compared post-hoc using Tukey's multiple comparison tests. We conducted statistical analyses in SAS 9.4 (SAS Institute 2011). Growth characteristics in our experiment were typically highly correlated, therefore we limit in-text figures to aboveground biomass, belowground biomass, and leaf C:N ratio, which are reasonable summary figures.

Results

Differences Among Species

In the absence of competition, the four species differed significantly in above- and belowground traits; however, we found no general trend separating the native and exotic species (Fig. 1). Differences among species were much more pronounced in the second temporal block, in which all species were more productive than in the first block (significant Species x Block interaction). Both native and exotic *Rubus* species grew larger than either *B. thunbergii* or *V. dentatum*. While the total stem length measured for *V. dentatum* was significantly smaller than the other three species, there was no significant difference between the aboveground biomass measured for *B. thunbergii* and *V. dentatum. B. thunbergii* had the smallest belowground biomass. The maximal root lengths for both *B. thunbergii* and *V. dentatum* were significantly shorter than those measured for both *Rubus* species. Only two responses showed a trend in which both exotic species differed from both native species; exotics produced more leaves and greater total fine root length than natives on average.

Species also differed in leaf C:N ratio and leaf ^{15}N enrichment and ^{13}C fractionation (Fig. 1). Differences among species were more pronounced in the second temporal block, as a result of greater growth in all species. *V. dentatum* displayed a significantly lower ^{15}N enrichment in leaf tissue than did the other three species. *V. dentatum* had greater water use efficiency than *B. thunbergii* and *R. allegheniensis* as indicated by ^{13}C fractionation of the leaves. *B. thunbergii* had a lower leaf C:N ratio than the other three species.

Effects of root competition on focal plant growth

Belowground competition reduced the growth of *Berberis* although the effect was dependent on temporal block (Fig. 2). Competition with either *Rubus* species reduced the aboveground biomass and total stem length of *B. thunbergii* more than other species. *V. dentatum* had an intermediate effect on *Berberis* size, while there was no detectable effect of intraspecific competition. Competitors did not affect belowground biomass, maximal root length, or fine root production of *B. thunbergii*.

For *R. allegheniensis*, intraspecific competition and competition with *R. phoenicolasius* reduced aboveground growth measures similarly, while competition with *B. thunbergii* or *V. dentatum* had no measurable effect on *R. allegheniensis* growth with no block by competitor interaction (Fig. 3). We found no differences among treatments on belowground growth metrics in this species.

R. phoenicolasius growth was also affected only by conspecifics or congeners irrespective of temporal block (Fig 4). Overall, intraspecific competition decreased *R. phoenicolasius* growth most strongly. *R. allegheniensis* had a somewhat weaker competitive effect than conspecifics on aboveground biomass and total leaf area. *B. thunbergii* and *V. dentatum* had no detectable effects on *R. phoenicolasius*.

For *V. dentatum*, temporal block interacted significantly with competitor, but both *Rubus* species reduced *V. dentatum* total stem length and aboveground biomass, while neither *B. thunbergii* nor conspecifics affected its growth (Fig. 5). Belowground growth was unaffected by competition.

Effects of root competition on focal plant leaf tissue chemistry

Competitors significantly reduced ^{15}N enrichment for *B. thunbergii* in all treatments except *R. allegheniensis.* Competition did not affect ^{13}C fractionation. C:N ratios were highest for plants competing with *R. phoenicolasius,* intermediate for plants competing with *R. allegheniensis*, and lowest for plants competing with *V. dentatum* (Fig. 2).

R. allegheniensis grown with *V. dentatum* showed significantly higher enrichment with ^{15}N compared to plants grown with conspecifics, but there were no differences among remaining treatments. ^{13}C fractionation was unaffected by competition; we did, however, find that competition with *R. phoenicolasius* resulted in a significantly higher C:N ratio compared to controls (Fig. 3).

For *R. phoenicolasius*, competition had no effect on ^{15}N enrichment or C:N ratio (Fig. 4). Intraspecific competition significantly enriched *R. phoenicolasius* in ^{13}C relative to controls (Appendix Fig. 14).

Leaf tissue chemistry in *V. dentatum* differed among blocks. Leaf ^{15}N enrichment and ^{13}C fractionation were unaffected by root competition, although root competition with both *Rubus* species significantly increased the leaf C:N ratio of *V. dentatum* plants (Fig. 5). Temporal block did not interact significantly with competitor for any of the four species.

Discussion

Observations of exotic species in their non-native ranges often suggest that exotic invasive species are superior competitors resulting from traits that influence nutrient uptake, allelopathy and growth patterns (Gioria & Osborne 2014). We found no consistent difference between native and exotic species in nitrogen acquisition or in their ability to affect nitrogen acquisition in a competitor plant, although we observed greater fine root production in the exotic species, *B. thunbergii* and *R. phoenicolasius*. Further, we did not observe any other consistent effects of these two exotic plants on competitor growth in our species.

Both *Rubus* species decreased competitor biomass by at least 50% relative to control plants, regardless of origin. It is perhaps unsurprising that the two *Rubus* species exhibited similar competitive effects given their phylogenetic relatedness, while the distantly related *B. thunbergii* and *V. dentatum* were distinctly different from one another in terms of the traits that we measured. In some invasions, escape from enemies may play a more prominent role than competition (Prior et al. 2015). Exotic species do not share an evolutionary history with naturally occurring pathogens and predators in their introduced range, enabling some exotic invasive species to reach high abundances in the introduced range. Since interactions with natural enemies can mediate competition among understory woody plants (Dietz *et al.* 2004; Ashton & Lerdau 2008; Knapp *et al.* 2008), enemy release could help to explain dynamics, namely the greater success of *R. phoenicolasius* compared to *R. allegheniensis*, not satisfactorily explained by competition in this study.

The presence of plant-soil feedbacks that develop over multiple growing seasons (Elgersma *et al.* 2011; Elgersma *et al.* 2012) and selective herbivory in field environments (Eschtruth & Battles 2009a, b) may help to explain why some shrubby species (*e.g.*, *B. thunbergii*), achieve competitive dominance in natural communities but did not exhibit belowground competitive superiority in our greenhouse experiment. We examined how belowground competition among recently established seedlings influenced shrub growth. Our field-collected soil was uniform across treatments and was not preconditioned by any of our species before the start of the experiment, making our experimental conditions most applicable to new invasions rather than to ongoing invasion by established shrubs.

The larger sizes of the two *Rubus* species are consistent with other empirical and theoretical work that larger plants are superior competitors (Goldberg 1987; Gaudet & Keddy 1988; Goldberg & Miller 1990). *R. phoenicolasius* and *R. allegheniensis* tended to be larger than either *B. thunbergii* or *V. dentatum*. The roots of larger plants fill a larger volume of soil, giving them greater access to resources via the larger volume of exploited soil. Both *Rubus* species exerted measurable competitive effects but did not routinely decrease leaf ^{15}N enrichment or root biomass of competitor plants, indicating that they did not consistently prevent competitors from acquiring nitrogen from a nutrient patch. The increased leaf C:N ratio of all species except *R. phoenicolasius* when grown with a *Rubus* competitor, however, suggests that the larger *Rubus* plants did decrease the total nitrogen available (but not ^{15}N) to smaller competitor plants.

Some *Rubus* species are strong competitors in disturbed areas and under resource limitation (Fotelli *et al.* 2002; Caplan & Yeakley 2013) and others are capable of becoming nuisance exotics (Ostertag & Verville 2002; Kosinski *et al.* 2014). Our results demonstrate that both *Rubus* can have measureable effects on plant performance through belowground competition, however, the mechanism by which they compete remains uncertain. We did not find evidence for consumptive competition for nitrogen in a nutrient patch, but allelopathy remains a potential mechanism driving these competitive interactions. Allelopathic potential has been documented in several *Rubus* species (Del Moral & Cates 1971; Cote & Thibault 1988; Balezentiene & Seziene 2010), but we did not examine the effect of growth-inhibitory compounds in this study. Multiple root competition mechanisms may be important in diverse biological communities and may simultaneously interact in complex or species-specific ways to determine competitive outcomes.

The interaction between the temporal block and competition treatment suggests that competition between plants was more intense and detectable in the second temporal block, when soil nutrient availability was greater and plants were actively growing, despite higher nitrogen limitation in the first block (Grime 1977; Li *et al.* 2010; Li *et al.* 2015). This interaction effect may also result from the seasonality of the species' growth patterns. Both *Rubus* species continued to grow actively throughout the autumn and winter seasons, suggesting the capability for extended autumn leaf phenology. On the other hand, *B. thunbergii* and *V. dentatum* remained dormant for several months despite our summer-like greenhouse temperature and light settings. Response to belowground competition by both *Rubus* species was unaffected by block (no interaction effect), while *B. thunbergii* and *V. dentatum* responded to belowground competition more strongly in the second temporal block. The effect of seasonal growth patterns in driving competitive outcomes may have important implications for shrub communities in which some shrub species exhibit plastic phenology. In warm autumns, such plants could gain a competitive advantage through an extended growing season compared to competitors (Fridley 2012), a situation possibly exacerbated by global climate change in temperate communities (Willis *et al.* 2010; Wolkovich *et al.* 2013; Caplan *et al.* 2015).

Conclusions

Root competitive ability contributes to a plant's total competitive effect and may influence competitive outcomes in shaded systems in which light is limited. Root competition occurred in our study system, but our results suggest plant size rather than plant origin determined competitive outcomes. Specifically, both species from the genus *Rubus* were superior competitors to *B. thunbergii* and *V. dentatum*. The two *Rubus* species were similar in size and had similar growth habits to one another, but differed significantly from the other two species. *B. thunbergii* and *V. dentatum* were unique in terms of the traits measured. Species in the *Rubus* genus grow quickly and their ability to spread rapidly via multiple vegetative mechanisms, as well as through seed dispersal by birds and mammals, contributes to their invasion potential in novel habitats (Lambrecht-McDowell & Radosevich 2005; Bennett *et al.* 2011). *R. phoenicolasius* was a slightly better competitor than *R. allegheniensis* in our experimental setting. Plant species that exhibit plastic growth phenology, such as those in the genus *Rubus*, may gain a competitive advantage during years with warmer autumn months by extending their growing seasons.

Figure 1.1a-c. All species responses of $\log_{10}$-transformed aboveground and belowground biomass, and leaf C:N ratio without competition.

Hatched bars correspond to exotic species and solid bars to native species. Dark bars are plants grown in the first block and light bars are plants grown in the second block. Different letters above bars indicate that treatments differ significantly.

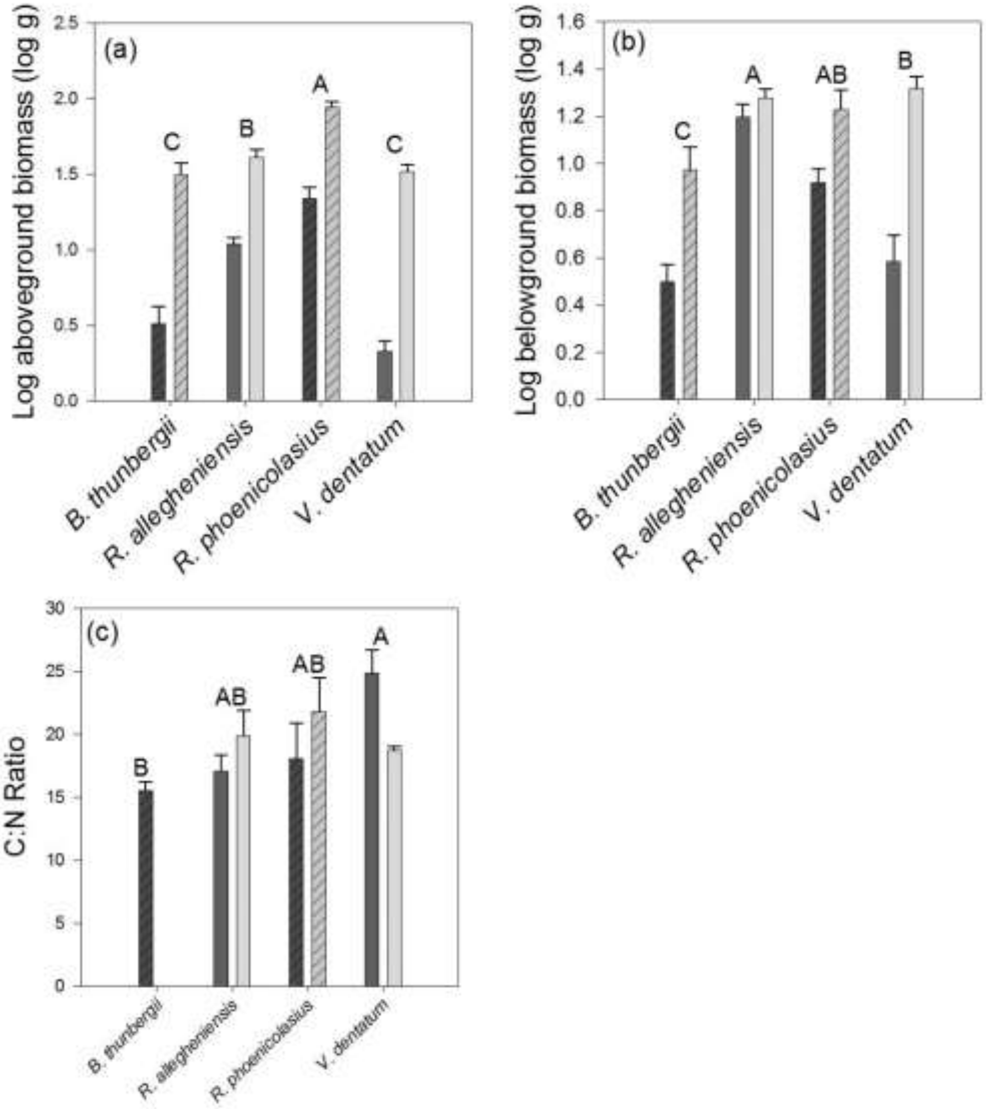


Figure 1.2a-c. ***B. thunbergii*** **$\log_{10}$-transformed aboveground and belowground biomass, and leaf C:N ratio to in response to competitor treatment.**

Hatched bars correspond to an exotic competitor, solid bars to a native competitor, and bars labeled control are plants grown without competitors. Dark bars are plants grown in the first block and light bars are plants grown in the second block. Different letters above bars indicate that treatments differ significantly.

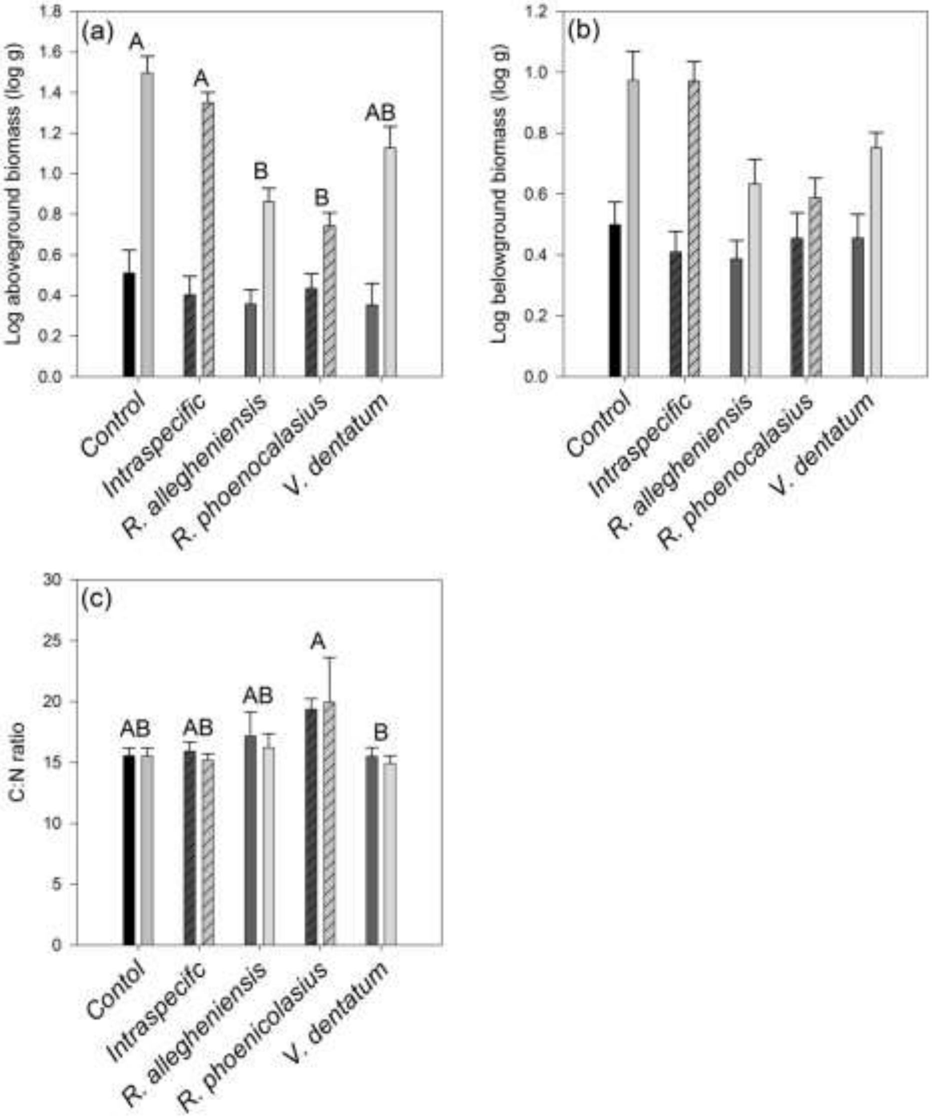


Figure 1.3a-c. *R. allegheniensis* log10-transformed aboveground and belowground biomass, and leaf C:N ratio to in response to competitor treatment.

Hatched bars correspond to an exotic competitor, solid bars to a native competitor, and bars labeled control are plants grown without competitors. Dark bars are plants grown in the first block and light bars are plants grown in the second block. Different letters above bars indicate that treatments differ significantly.

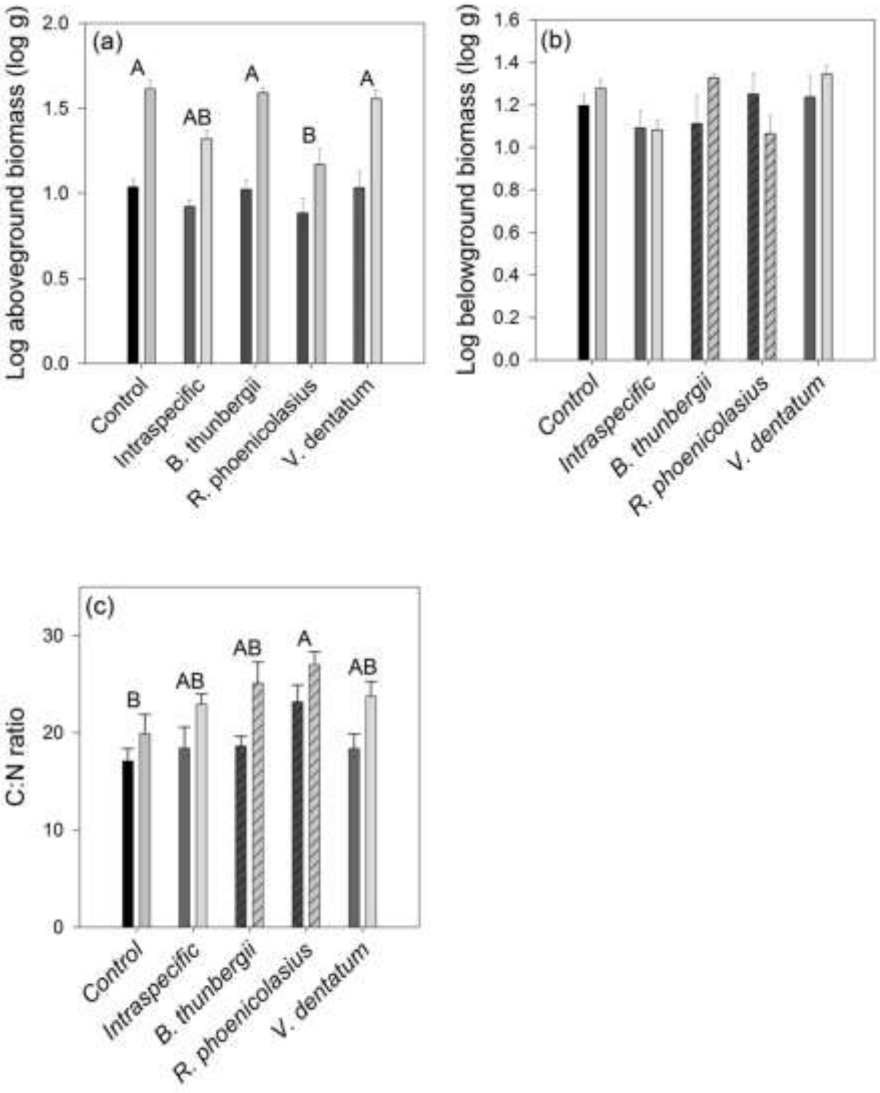


Figure 1.4a-c. *R. phoenicolasius* $\log_{10}$-transformed aboveground and belowground biomass, and leaf C:N ratio to in response to competitor treatment.

Hatched bars correspond to an exotic competitor, solid bars to a native competitor, and bars labeled control are plants grown without competitors. Dark bars are plants grown in the first block and light bars are plants grown in the second block. Different letters above bars indicate that treatments differ significantly.

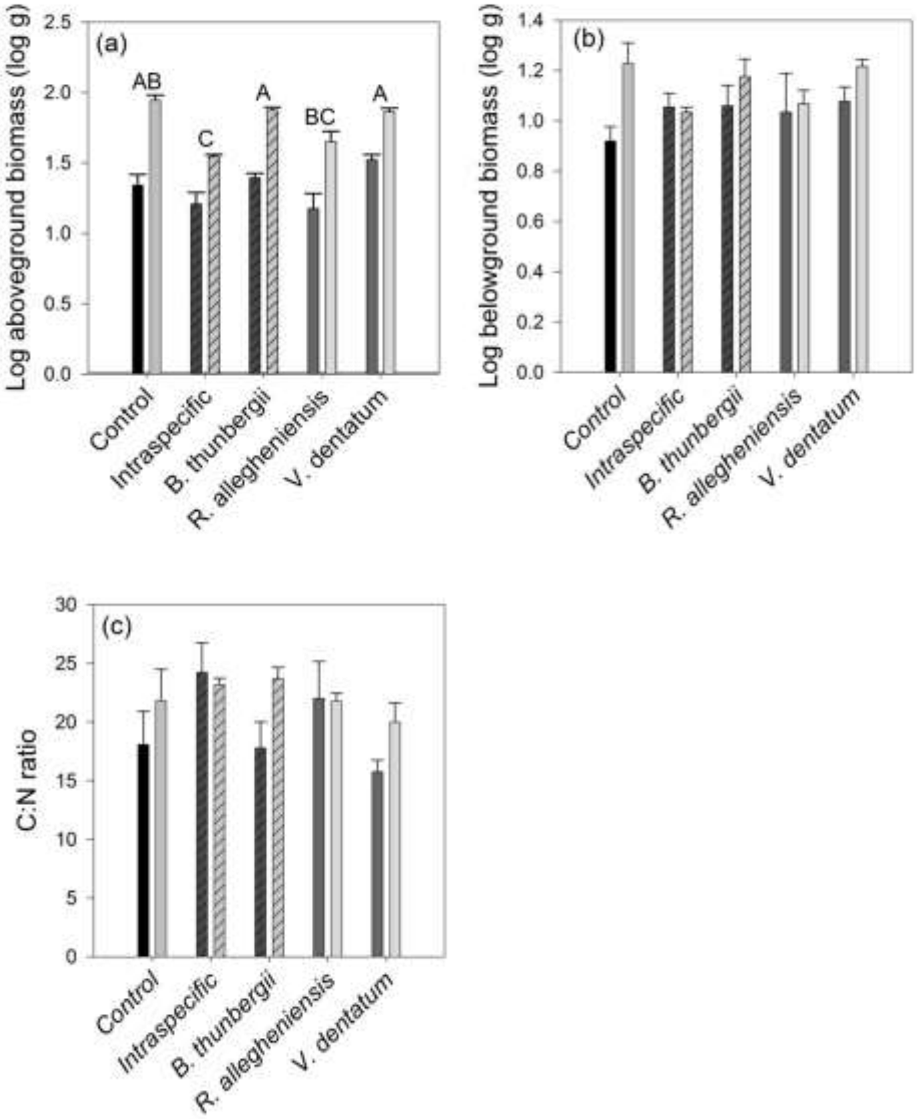
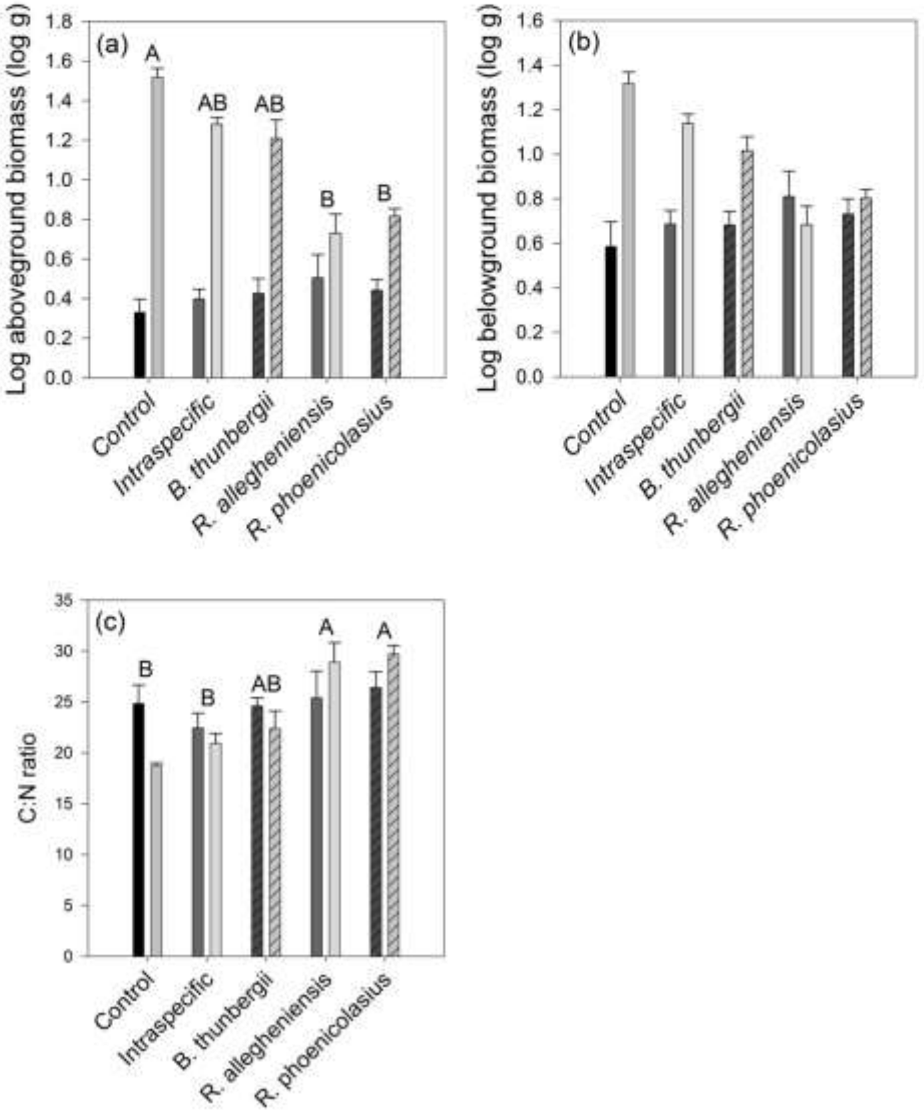


Figure 1.5a-c. *V. dentatum* log$_{10}$-transformed aboveground and belowground biomass, and leaf C:N ratio to in response to competitor treatment.

Hatched bars correspond to an exotic competitor, solid bars to a native competitor, and bars labeled control are plants grown without competitors. Dark bars are plants grown in the first block and light bars are plants grown in the second block. Different letters above bars indicate that treatments differ significantly.



CHAPTER 2

Evolution alters the consequences of invasions in experimental communities

Abstract

Evolution has the capacity to alter the course of biological invasions, though such changes remain mostly unexplored by experiments. Integrating evolution into studies of invasions is important, because species traits can potentially evolve in ways that either moderate or exacerbate the impacts of invasions. We assessed whether species evolved during experimental invasions by comparing the performance of founder populations and their potentially evolved descendants in communities of ciliates and rotifers. Residents (analogous to native species) having prior experience with invaders consistently reduced the performance of naïve invaders, supporting the emergence of increased biotic resistance as one consequence of evolution during invasions. Experienced invaders exhibited both increased and decreased performance depending on the invader species considered. Through its influence on performance and species abundance, evolution also changed community composition during the course of invasions. The idiosyncratic patterns of evolutionary changes in invading and resident species complicate predictions about the long-term consequences of invasions from initial post-invasion dynamics.

Key words: community ecology, eco-evolution, invasion ecology microevolution

Introduction

Biological invasions pose a major threat to biodiversity (Simberloff *et al.* 2013), yet research on the eco-evolutionary dynamics of biological invasions is still in its earliest stages (Lambrinos 2004; Strayer *et al.* 2006; Saul & Jeschke 2015). Eco-evolutionary dynamics can affect interspecific interactions (Palkovacs *et al.* 2009; Schoener 2011) in ways that influence invader success and native species' responses and modify the impacts of biological invasions (Carroll 2011). Studies of biological invasions are typically of short-duration (Strayer *et al.* 2006), and factors proposed to influence biological invasions largely overlook the possibility that evolution may alter the impacts of invasions over time (Facon *et al.* 2006; Saul *et al.* 2013).

Evolutionary changes (occurring over fewer than 100 generations) in morphology, behaviour, and genotype occur in both invaders and native species during biological invasions (Lee 2002; Strauss *et al.* 2006a; Colautti & Lau 2015). Previous studies generally concentrate on trait-based changes in focal species without linking evolutionary dynamics to changes at the community level through interspecific interactions (Lee 2002; Strauss *et al.* 2006a; Carroll 2007a; Carroll 2007b; Mealor & Hild 2007; Phillips *et al.* 2010; Goergen *et al.* 2011; Shine 2012; Strauss 2012). A small number of studies have tracked long-term population dynamics following invasions (Carlsson *et al.* 2010; Carthey & Banks 2015). They demonstrate the potential for evolution to alter interspecific interactions over long time scales, but they stop short of unambiguously attributing observed changes to ongoing evolution.

Strong evidence for evolution during invasions requires demonstration of stable changes in traits in response to an imposed selective regime. In the context of biological invasions, this requires measuring traits before and after invaders and residents have interacted for some time. Studying eco-evolutionary dynamics in invaded communities is especially challenging; resident and exotic species are often studied early in the course of interaction, before much evolution can occur, or much later, when species may have evolved, but initial trait states are no longer available for comparison. Often, the detailed history of introductions and spread for invading exotic species is poorly known (Le Roux & Wieczorek 2009; Kolbe *et al.* 2012), which further complicates inferences about potential evolution during the course of invasions. Ideally, measuring the traits of both resident and invading species early and later in the course of invasions can definitively assess whether evolution affects long-term invasion dynamics (Kolbe *et al.* 2012). Such comparisons are possible in laboratory communities composed of organisms with short generation times where evolution can occur over experimentally tractable time frames. Experiments using these systems can alter the duration of post-invasion interactions and manipulate opportunities for potential evolution (Warren *et al.* 2006).

We compared the performance of populations of resident and invading species before and after they had interacted, and potentially evolved, for approximately 200-400 generations. We used two different resident species assemblages (hereafter termed A and B) composed of aquatic bacteria, protists and rotifers. One species from each assemblage was designated as an experimental invader of the other. Assemblage A contained five ciliates and a rotifer, and assemblage B contained three different ciliates and a different rotifer (Methods and Supplementary Figure 1). *Paramecium bursaria* (a resident in assemblage A) and *Euplotes daidaleos* (a resident in assemblage B) were selected as invaders that were experimentally introduced into the other assemblage (Methods and Fig. 1). The designated invaders were functionally similar (mixotrophic bacteriovores containing symbiotic chlorellae) and the resident assemblages that they invaded contained congeners (*Euplotes patella* and *Paramecium caudatum*) with which they might be expected to interact strongly. Theory suggests that the consequences of evolution between invaders and natives may depend on the type of interspecific interaction involved (Abrams 1996; Jones & Gomulkiewicz 2012), even leading to reduced performance or extinction (Rummel & Roughgarden 1983) under some circumstances.

Our experiment addressed three general questions. 1) Does evolution alter population abundances and community composition when one species invades a community? 2) Are patterns of changed abundance consistent with altered biotic resistance of residents or invasiveness of invaders? 3) Are evolutionary responses idiosyncratic or consistent across different communities and invaders? Post-invasion abundance was the trait used to assess performance and infer evolution in experimental treatments with different interaction histories (Fig. 1). By varying whether or not invaders and residents interacted for many generations before assessing performance in a second round of invasion, we determined whether performance changed over time. Treatments differed in the experienced (*i.e.*, those that have a recent interaction history of approximately 200-400 hundred protist generations) or naïve status (*i.e.*, no recent interaction history) of invading and resident species: 1) invading and resident species both experienced, 2) experienced invaders and naïve residents, 3) invaders and residents both naïve, 4) naïve invaders and experienced residents, and 5) an uninvaded control containing only resident species originally present before invasions. We compared abundances of species against multiple *a priori* predictions for both invaders and residents (Supplementary Figure 2). We expected that: 1) If prolonged interaction resulted in increased invader performance, experienced invaders (+I) would outperform naïve invaders (-I) when grown with naïve residents (-R) (+I/-R > -I/-R, Fig. 1a), and residents (+R or -R) would have lower abundances when grown with experienced (+I) vs. naïve invaders (-I) (+R/+I < +R/-I and -R/+I < -R/-I Supplementary Figure 2). 2) If experience increased resident performance, naïve invaders (-I) grown with experienced residents (+R) would have lower abundances compared to those grown with naïve residents (-R) (-I/+R < -I/-R, Fig. 1b). We expected the abundances of experienced residents (+R) to be higher than naïve residents (-R) when interacting with experienced or naïve invaders (+I or -I) (+I/+R > +I/-R and -I/+R > -I/-R, Supplementary Figure 2).

Methods

Experimental Design

Overview

Our experimental protocol used protists and rotifers with short generation times so that evolutionary responses to different selective regimes imposed by different invasion scenarios could be measured over tractable periods of time. The experiment involved three phases to ensure that populations of species had ample time for potential evolutionary change and possible coadaptation to be measured. Phase one ensured that the species grown in each assemblage, A and B, had sufficient time to interact and potentially coadapt to one another, thus sharing a recent history of interaction that spanned 300-550 protist generations. Phase two established the actual invasion of replicates of each assemblage by an invader drawn from the other community. Species in the invaded treatment interacted for a sufficient number of generations (approximately 200-400 protist generations) so that the invaders and species in the resident communities (analogous to native species with a shared history of interaction and potential coadaptation) might have time to potentially evolve. Phase three compared the performance of different lines of potentially evolved invaders and residents to naïve invaders and residents in all possible combinations to assess the effects of interaction history on species performance and community composition. Because the treatments within each community differed solely in terms of the length of interaction history, rather than species composition or external environmental factors, phenotypically plastic responses would be consistent across treatments within a community (and likely transient in nature). If the observed differences among treatments did involve an inducible defence it would imply some sort of evolutionary change drove differences in plastic traits. A detailed description of the experimental design follows.

Phase 1

The original "native" species, termed residents, in each source assemblage consisted of naturally co-occurring bacterivorous and predatory ciliates and rotifers originally collected from the same pond ("Bamboo Pond") on the campus of Rutgers University. Assemblage A contained five ciliates and a rotifer, *Blepharisma americanum*, *Spirostomum teres*, *Euplotes patella*, *Prorodon niveus*, *Paramecium bursaria*, and *Lecane* sp.. Assemblage B contained three ciliates and a second rotifer, *Stentor coeruleus*, *Paramecium caudatum*, *Euplotes daidaleos*, and *Monostyla* sp.. These assemblages were selected for their stability in species composition and were maintained in laboratory cultures for three years, equal to at least 550 (slowest growing protist species) to 1095 (fastest growing protists) protist generations and approximately 275 generations for rotifers, before the start of the experiment. *Blepharisma americanum*, *Spirostomum teres*, *Euplotes patella*, *Prorodon niveus*, and *Lecane* sp. were grown continuously together during this period, as were *Stentor coeruleus*, *Paramecium caudatum*, and *Monostyla* sp. Each invader was maintained in separate laboratory cultures from first collection until several months before the start of phase 1 of the experiment, at which time they were added to their respective source assemblages (*P. bursaria* to assemblage A and *E. daidaleos* to assemblage B). We initiated our experiment using our two different assemblages, A and B to provide 18 months, or about 300 to 550 protist generations, in which species in each assemblage further coadapted to laboratory conditions and one another (Fig. 1, phase 1). In this way we ensured a prolonged period of recent evolutionary history for each assemblage maintained separately in the lab. Documented microgeographic evolution suggests that even within a single geographic range of a population, different sub-populations of a species can differ both genetically and in terms of their interactions within a community, suggesting that the prolonged period of laboratory culture that our assemblages experienced allopatrically would allow them to potentially interact differently depending upon our experimental treatment. If the prolonged recent evolutionary history in the lab was insufficient to overcome the previous history before collection in Bamboo Pond, we would not expect to see an effect of our treatment. Assemblages were initially inoculated with the same four bacterial species, *Proteus vulgaris, Serratia marcesens, Bacillus subtilis,* and *Bacillus cereus*, as known edible resources for the bacterivores, but we did not quantify the number and abundances of bacterial species and others were likely present. Five treatments manipulated the duration of interaction history following invasion and possible evolution for assemblage A and B, for a total of 10 treatments, each replicated five times in separate microcosms.

Microcosms were loosely lidded 250mL jars with 100 mL of medium (1 Protist Pellet, Carolina Biological Supply plus 0.14 mg Herptivite vitamin supplement in 2.8 L of well water, autoclaved before addition of organisms) and two sterile wheat seeds for additional nutrients. This phase created the starting conditions for each assemblage subsequently subjected to experimental invasions. Communities were subcultured every 3 weeks by gently mixing each jar and then adding approximately 3 mL of old culture to a new jar with fresh medium. To ensure that bacterial resource species remained consistent throughout the experiment, fresh medium was bacterized with the same stock strains of bacteria consistently grown individually in the absence of bacterivores. Bacteria in fresh medium grew for three days at room temperature and were highly abundant before the addition of 3 mL of old culture containing, ciliates, rotifers, and bacteria. Jar location was randomized in a Percival incubator at 22° C with a 5 hour light / 19 hour dark photoperiod. Counts of the number of individuals in subsamples of known volume from each jar estimated species densities weekly.

Phase 2

The second phase of the experiment involved invading replicates of each assemblage of resident communities, A and B, with an invader drawn from the other assemblage, to create a situation in which the invaders and resident species had an opportunity to potentially evolve. We experimentally invaded 5 replicates of resident community A with 15-20 cells of *E. daidaleos* originally grown in source community B, and resident community B with 15-20 cells of *P. bursaria* originally grown in source community A (Fig. 1, Phase 2, +I/+R treatments). Previous experience with these species indicated that this number of introduced individuals is sufficient to establish populations. We chose these mixotrophic species as invaders (both contain photosynthetic symbionts) because they are functionally similar. Both also invaded assemblages in which another congener was already established. This approach achieved some potential degree of niche separation between resident and invading species, while still allowing for interspecific interactions among organisms. Following these initial invasions, communities grew for another 13 months, or approximately 200-400 protist generations (Fig. 1, Phase 2), allowing invaders and residents to potentially evolve over time. At the same time, replicates of the original uninvaded assemblages remained a source of “naïve” species without a history of post-invasion interaction (Fig. 1).

Phase 3

The third phase involved testing whether populations that had an opportunity to evolve for 200-400 protist generations after invasion differed in performance from populations that did not have an opportunity to evolve. We established invasions during a three-week period to create the final treatments used to evaluate the performance of naïve or potentially coevolved sets of species (Fig. 1, Phase 3). For –I/+R treatments (naïve invader/experienced residents), we first isolated inocula of invasion-experienced resident species (from +I/+R treatments for each assemblage) and reassembled them into communities with the original resident species composition for A and B. Next we invaded them with an inoculum of invaders derived from populations of naïve invaders. For each of the invasions, we again used an inoculum of 15-20 invading cells per replicate. Similarly, freshly subcultured assemblages of naïve residents were inoculated with invaders derived from experienced (+I) or naïve (-I) populations (with or without a long history of interaction with members of the invaded assemblages). All treatments were subsequently checked for successful establishment of the invaders. To assess the effect of interaction history on post-invasion community dynamics, we again estimated the abundances of all species weekly for 6 weeks.

Statistical analyses

We calculated species densities as number +1 per mL and then $\log_{10}$-transformed the values before analysis. We then averaged $\log_{10}$-transformed densities over the six-week time period for each replicate following the final invasions to capture the post-invasion dynamics. These mean values became the response variables for our statistical analyses. For each assemblage, A and B, a Multivariate Analysis of Variance (MANOVA SAS 9.4 (SAS Institute 2011)) evaluated the effect of potential evolutionary treatments on the mean of the log of each resident/native species' abundance. In assemblage A, one native species, *P. niveus*, failed to establish in the –I/+R treatment, likely because of exceptionally small propagule size resulting from the difficulty of locating *P. niveus* individuals in the experienced invader/experienced residents (+I/+R) treatment when we established the –I/+R treatment. For this resident/native species and each invader, we instead conducted separate ANOVAs for dynamics. ANOVAs testing the response of invaders included the "source" community treatment to determine how evolutionary experience affected performance relative to naïve invaders in their source community. We evaluated significant differences between treatment means for each response variable using Tukey's HSD multiple comparison test (at $\alpha = 0.05$) to help identify which treatments differed in abundance.

To evaluate the overall community response to differing evolutionary histories of invaders and resident species, we conducted Principal Component Analyses (PCAs) of $\log_{10}$-transformed mean species abundances for each assemblage for post-invasion composition. For assemblage A, native species *P. niveus* was not included in the PCA to prevent its lack of establishment in the –I/+R treatment from unduly affecting the analysis on community composition. We then used community scores for the first three principal components as response variables in separate MANOVAs for each assemblage for post-invasion composition.

Results

A post-invasion history of interaction with resident species changed the performance of both invaders, *E. daidaleos* invading community A ($F_{3,16} = 97.25$, $p < 0.0001$, see Methods) and *P. bursaria* invading community B ($F_{3,16} = 54.80$, $p < 0.0001$). *E. daidaleos* performance increased when populations were founded with individuals having a previous history of interaction with residents (Fig. 2a,c). The highest mean abundance occurred in the experienced invader/experienced residents treatment (+I/+R). Experience with the residents of assemblage A also improved the performance of *E. daidaleos* over that in its source community, assemblage B (Fig 2c). *P. bursaria* performance decreased when interacting with experienced residents (Fig. 2b,d), suggesting that changes in the residents of assemblage B resulted in increased biotic resistance to invaders. Changes in *P. bursaria* performance after interacting with residents of the invaded assemblage also reduced its average abundance relative to that in its source community, assemblage A (Fig. 2d). We also noted that initial invasion by naïve *P. bursaria* into an experienced resident assemblage (-I/+R) required roughly twice the propagule pressure of invaders and lower abundances of resident species relative to invaders in other treatments.

Interaction history also influenced the abundance of resident species in both communities (assemblage A: $F_{20,54} = 16.43$, $p < 0.0001$, assemblage B: $F_{16,52.6} = 3.80$, $p = 0.0001$, Methods), with responses varying idiosyncratically among species (Figs 3a-f, 4a-d). Four species in assemblage A (*Blepharisma americanum, Euplotes patella, Prorodon niveus,* and *P. bursaria*) became less abundant after prolonged interactions with an invader, while one species from assemblage A (*Spirostomum teres*) and three from assemblage B (*Stentor coeruleus, Paramecium caudatum,* and *E. daidaleos*) became more abundant after prolonged interactions with an invader. Interaction history did not affect the performance of either rotifer species. The collective changes in species abundances associated with different interaction histories changed the overall species composition of both assemblages (Methods). In assemblage A, communities composed of experienced invaders and residents (+I/+R) had elevated abundances of *E. daidaleos* and *S. teres* relative to other treatments. In the remaining treatments assemblage A had higher abundances of *B. americanum* and *E. patella* (Fig. 5a). For assemblage B, the two treatments with naïve residents (+I/-R, -I/-R) were most similar in composition (Fig. 5b). These treatments had higher abundances of one resident species, *Monostyla* sp., and the invader, *P. bursaria,* while treatments with experienced residents had higher abundances of *S. coeruleus* and lower abundances of the invader *P. bursaria*.

Discussion

The history of interactions altered the performance of both resident and invading species in our experiment. We propose that evolution, rather than phenotypic plasticity (*sensu* DeWitt & Scheiner 2004) led to observed differences in performance. Within assemblages, treatments differed only in the duration of potential preadaptation of the invaders and natives, and not in the identities of species present (DeWitt & Scheiner 2004). Attributing differences to phenotypic plasticity would require that: 1) different plastic (non-genetic) responses were induced by the same set species, with differences depending only on the history of interaction, and 2) that those plastic responses would persist and be transmitted over many generations of population growth, long after the differences in inducing conditions had ended. Studies of other organisms, including a ciliate, document that rapid evolutionary responses to interaction history and species identity can occur (Urban 2010; Hiltunen & Becks 2014; Zuppinger-Dingley *et al.* 2014), supporting the likelihood of rapid evolution (*sensu* Hairston *et al.* 2005) in our system. The persistent differences seen among our treatments are consistent with the kinds evolutionary changes in performance documented by quantitative genetic techniques (Falconer 1960) in other species.

Species in our assemblages can potentially interact directly and/or indirectly via multiple food web pathways (see Supplementary Figure 1); consequently, we cannot unambiguously attribute altered performance to changes in traits such as competitive ability, antipredator defence, or attack rates. At least two resident species, *E. patella* and *P. niveus*, became less abundant in the treatment containing experienced invaders and residents (+I/+R), so much so that they became difficult to detect (Fig. 3c, e). This result would not have been apparent if only the naïve invasion treatment (-I/-R), analogous to recent invasions in nature, was compared to the uninvaded resident community (control treatment, -R). This result underscores the importance of considering long-term eco-evolutionary dynamics in biological invasions, since some responses may take many generations to become apparent.

Evolution can potentially enhance the performance of invaders and/or increase the resistance of resident species. Evolution of increased invader performance (*e.g., E. daidaleos* invading assemblage A) can exacerbate the impact of invasions over time. Other invaders may become less successful over time, if increased invader performance is outstripped by increased resident performance (*e.g.*, assemblage B), leading to the appearance of increased biotic resistance. The amount of existing genetic variation and the strength of selection on traits that affect performance can both influence these differences in evolutionary potential. The long-term consequences of evolution among invaders and residents may be difficult to predict from early post-invasion patterns(Koch *et al.* 2014), but our findings suggest that eco-evolutionary dynamics may alternately exacerbate or reduce community-level impacts over time. Because most biological invasions are difficult or impossible to reverse, it is important to recognise that phenotypic changes will sometimes ameliorate the impacts of invaders without human intervention, while other invasions will require continued intervention to minimise their impacts. The challenge remains in predicting when initial impacts of invasions will become more or less severe over time.

Figure 2.1. Experimental design with two assemblages, A and B.

A species designated as an invader was first grown as a species in one assemblage (the source) in Phase 1, *e.g. P. bursaria* in A, before being introduced into the other resident assemblage, B (Phase 2). In the final phase of the experiment, Phase 3, in addition to uninvaded source assemblages (S) used as experimental controls, potentially evolved and naïve invaders and resident assemblages were paired in all possible combinations to evaluate interactions among species (sample size n = 5 for each combination of invader and resident assemblage). Treatments are coded by whether invaders (I) and species in the resident assemblage (R) have had the opportunity to coevolve (+) or not (-) through the presence or absence of a prolonged history of recent interaction (approximately 200 generations). Green arrows designate transfer of resident communities and gold arrows designate transfer of invaders. Dark arrows originate from the evolved lines while light arrows originate from the uninvaded lines.

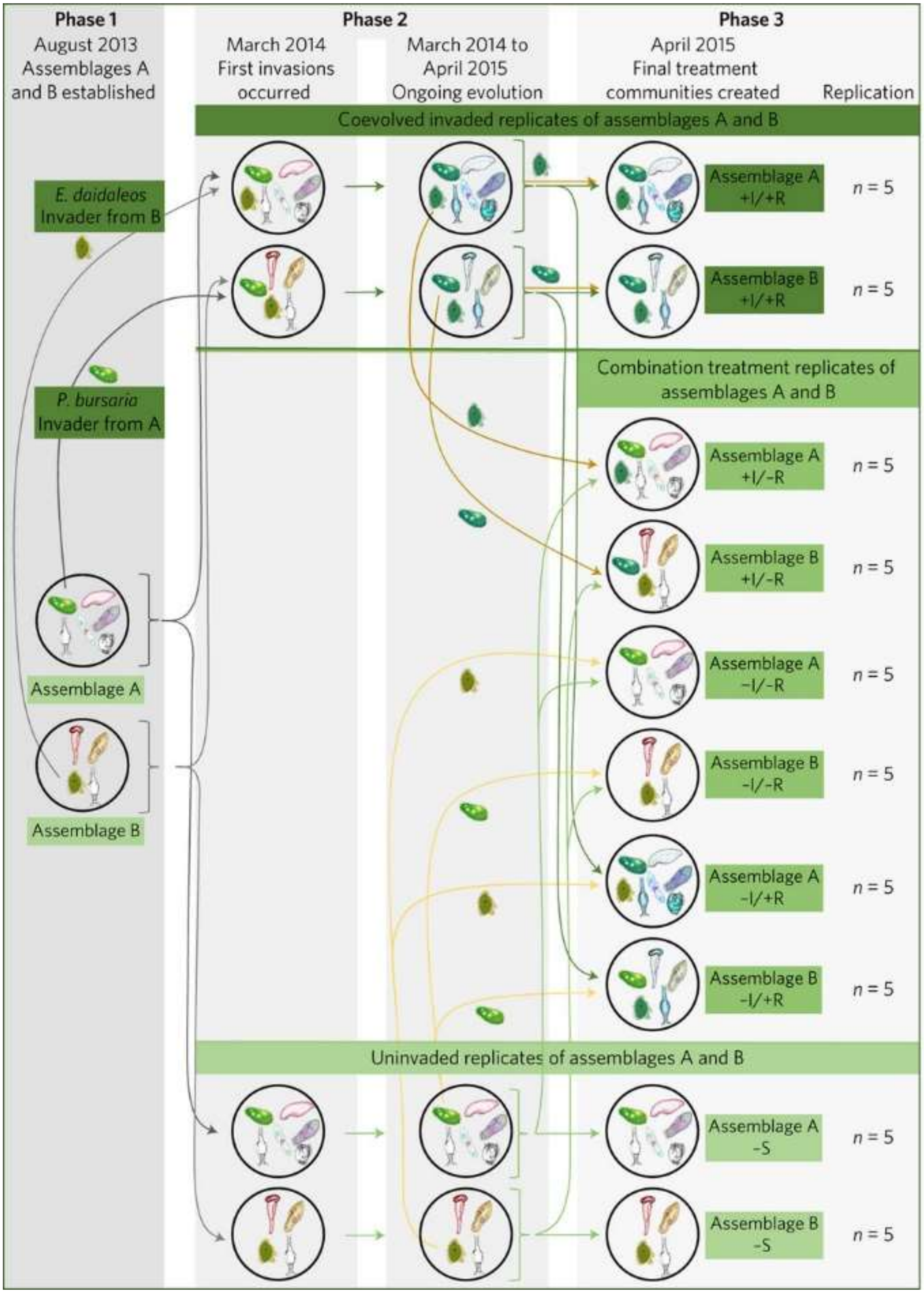

Phase 1
August 2013
Assemblages A
and B established
Phase 2
March 2014
First invasions
occurred
March 2014 to
April 2015
Ongoing evolution
Phase 3
April 2015
Final treatment
communities created
Replication
Coevolved invaded replicates of assemblages A and B
E. daidaleos
Invader from B
P. bursaria
Invader from A
Assemblage A
+I/+R
n = 5
Assemblage B
+I/+R
n = 5
Combination treatment replicates of
assemblages A and B
Assemblage A
+I/-R
n = 5
Assemblage B
+I/-R
n = 5
Assemblage A
-I/-R
n = 5
Assemblage B
-I/-R
n = 5
Assemblage A
-I/+R
n = 5
Assemblage B
-I/+R
n = 5
Assemblage A
Assemblage B
Uninvaded replicates of assemblages A and B
Assemblage A
-S
n = 5
Assemblage B
-S
n = 5

Figure 2.2a-d. Prediction and response to potential evolutionary history by both invaders.

a,b predictions and observed responses c, for *E. daidaleos* (n = 5) and d, for *P. bursaria* (n = 5). Treatment groups are labelled on the x-axis with a "+" or "-" for experienced or naïve respectively, while an "I" or "R" refers to the invader or species in the resident community, respectively, while "S" refers to species from the source community. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different letters above the boxes. Error bars indicate ±1 standard error of the mean. In a, symbols indicate relative positions of the means (rather than absolute values) that might result if invaders evolved improved performance while species from the resident assemblage did not. In b, symbols indicate relative differences in a different scenario, in which species from the resident assemblage evolved improved performance, while invaders did not. *A priori*, either scenario is possible. Our predictions implicitly assume that if species are not evolving, then the "+" and "-" treatments are actually equivalent. Thus, for example, if only the invader evolves increased performance (a), it will perform equally well with +R or –R resident species because no evolution has occurred among the species in the resident assemblage, making these groups functionally equivalent. The complete set of *a priori* predictions are presented in Supplementary Information Figure 2.

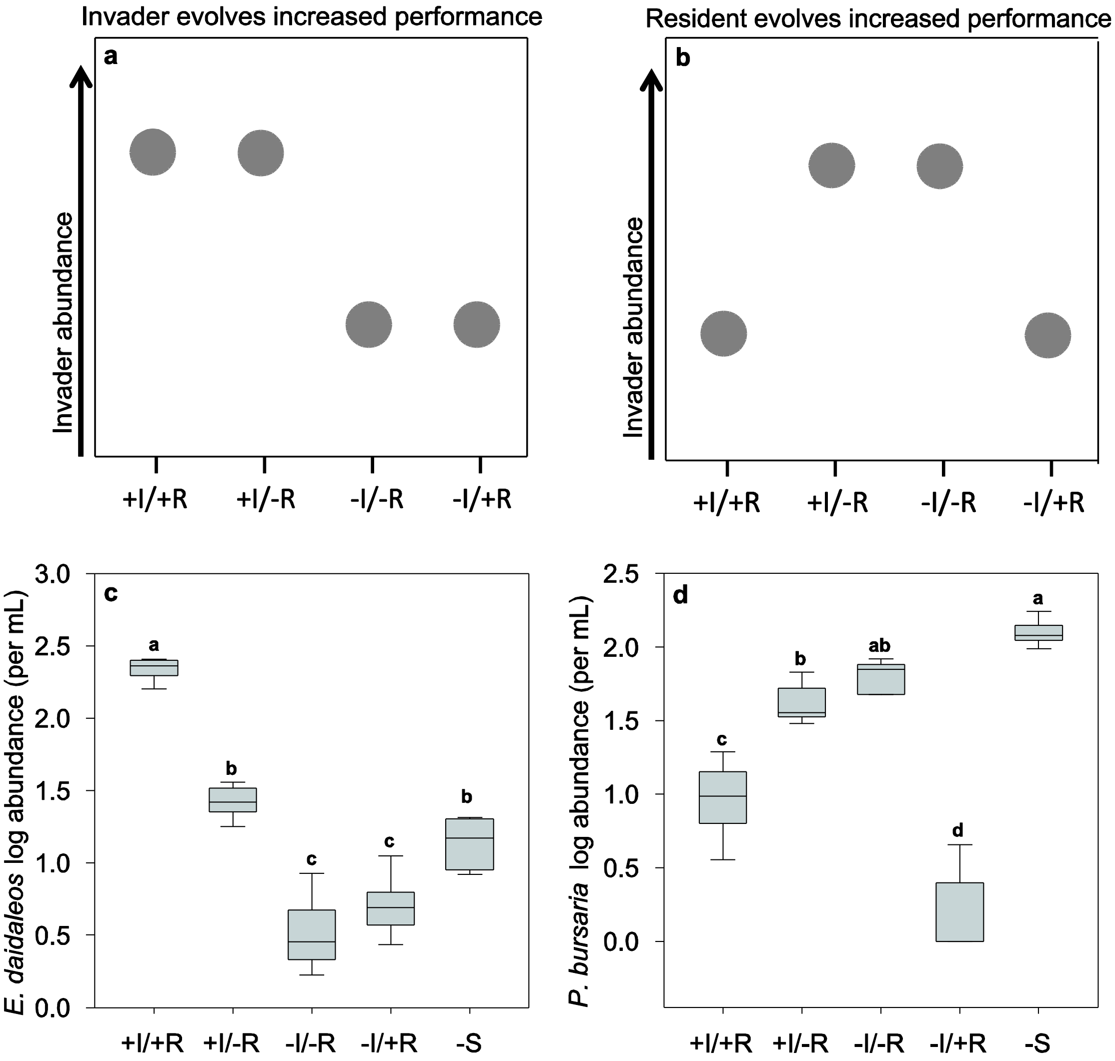

Invader evolves increased performance
a
Invader abundance
+I/+R
+I/-R
-I/-R
-I/+R
Resident evolves increased performance
b
Invader abundance
+I/+R
+I/-R
-I/-R
-I/+R
c
E. daidaleos log abundance (per mL)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
c
c
b
+I/+R
+I/-R
-I/-R
-I/+R
-S
d
P. bursaria log abundance (per mL)
2.5
2.0
1.5
1.0
0.5
0.0
c
b
ab
d
a
+I/+R
+I/-R
-I/-R
-I/+R
-S

Figure 2.3a-f. Response by resident species of assemblage A to potential evolutionary history with the invader, *E. daidaleos*.

Treatment groups are labelled on the x-axis with a "+" or "-" for evolved or naïve respectively, while an "I" or "R" refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Error bars indicate ±1 standard error of the mean.

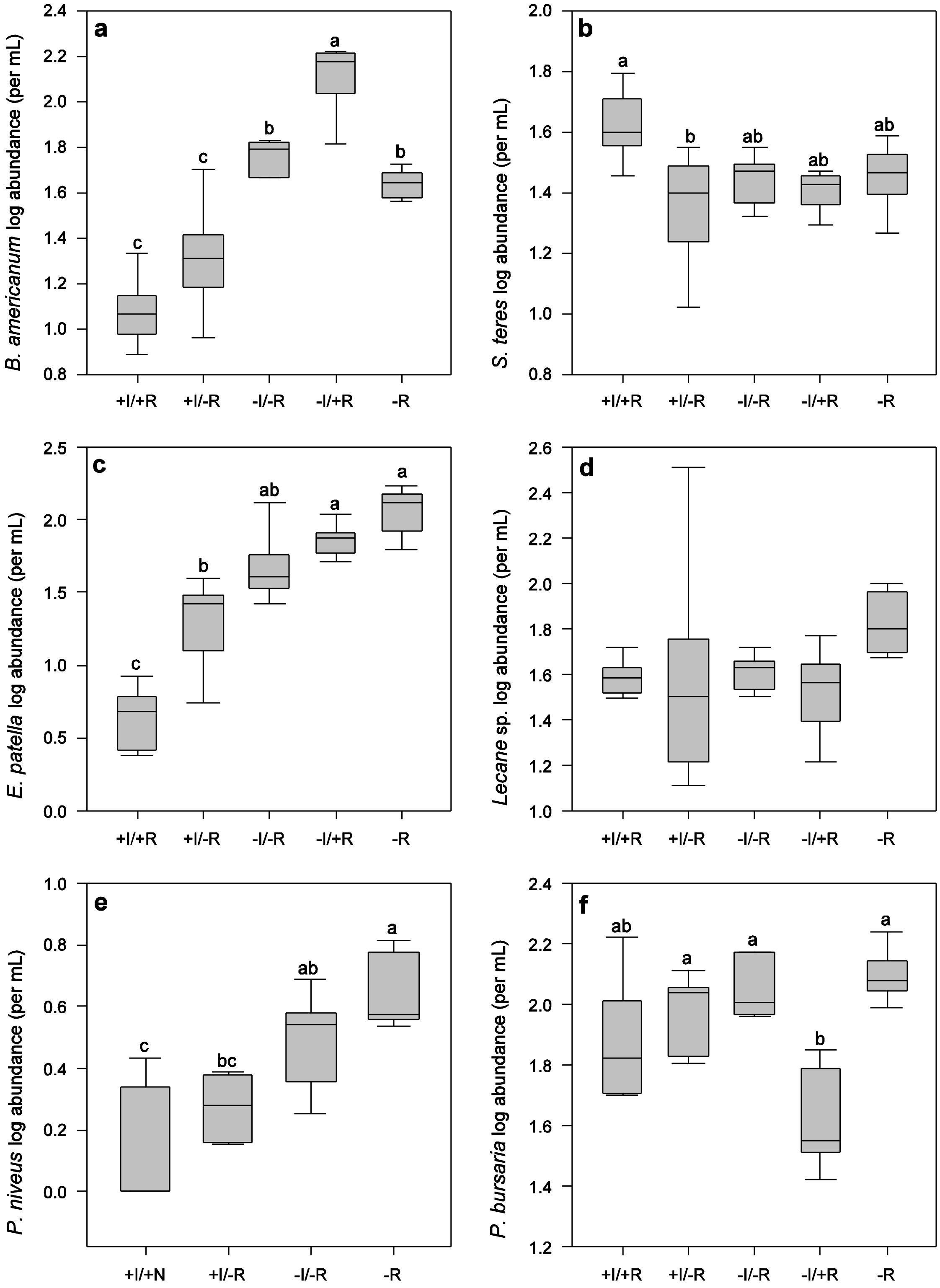

a
B. americanum log abundance (per mL)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
c
c
b
a
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
S. teres log abundance (per mL)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
a
b
ab
ab
ab
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
E. patella log abundance (per mL)
0.0
0.5
1.0
1.5
2.0
2.5
c
b
ab
a
a
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Lecane sp. log abundance (per mL)
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
+I/+R
+I/-R
-I/-R
-I/+R
-R
e
P. niveus log abundance (per mL)
0.0
0.2
0.4
0.6
0.8
1.0
c
bc
ab
a
+I/+N
+I/-R
-I/-R
-R
f
P. bursaria log abundance (per mL)
1.2
1.4
1.6
1.8
2.0
2.2
2.4
ab
a
a
b
a
+I/+R
+I/-R
-I/-R
-I/+R
-R

Figure 2.4a-d. Response by resident species of assemblage B to potential evolutionary history with the invader, *P. bursaria*.

Treatment groups are labelled on the x-axis with a "+" or "-" for evolved or naïve respectively, while an "I" or "R" refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Error bars indicate ±1 standard error of the mean.

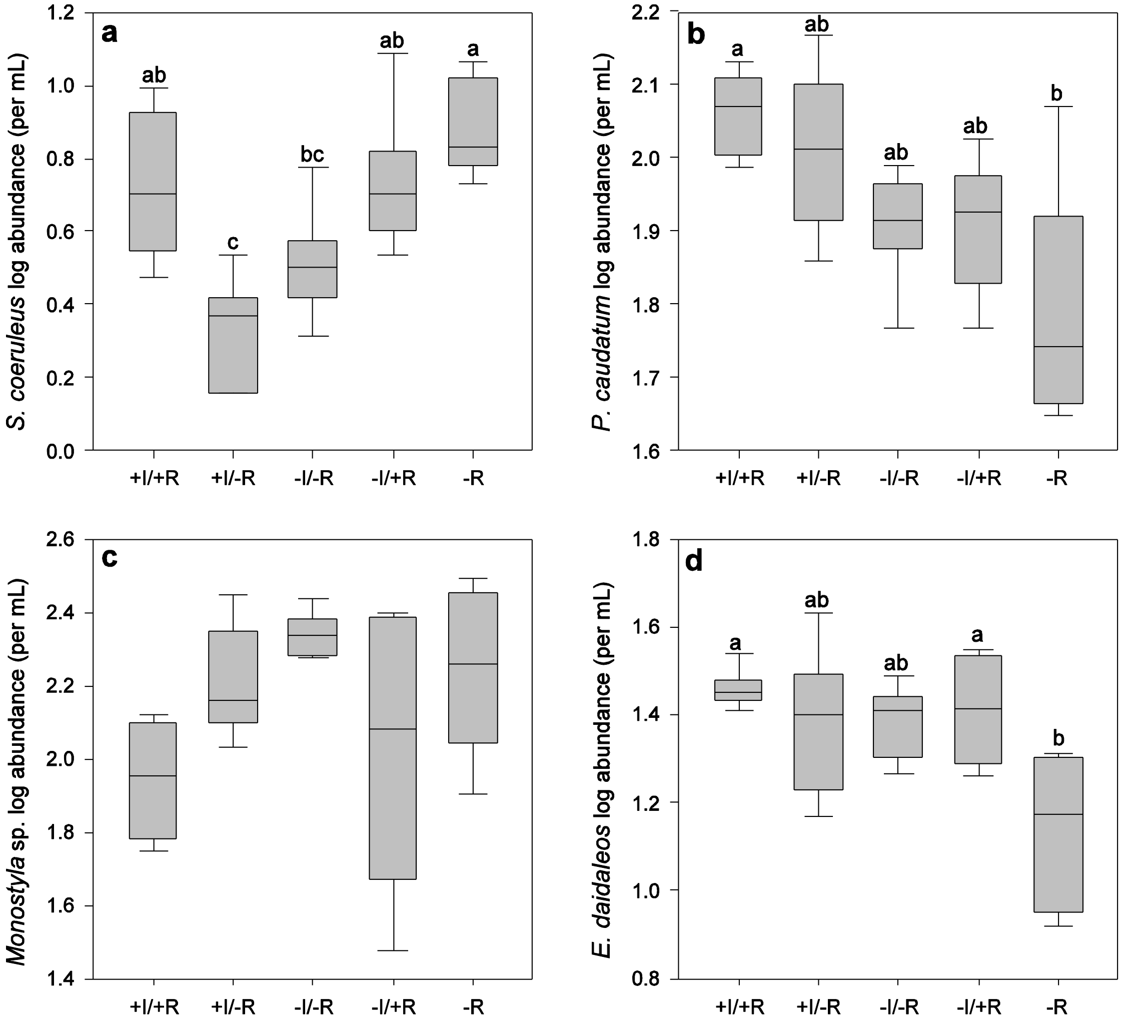
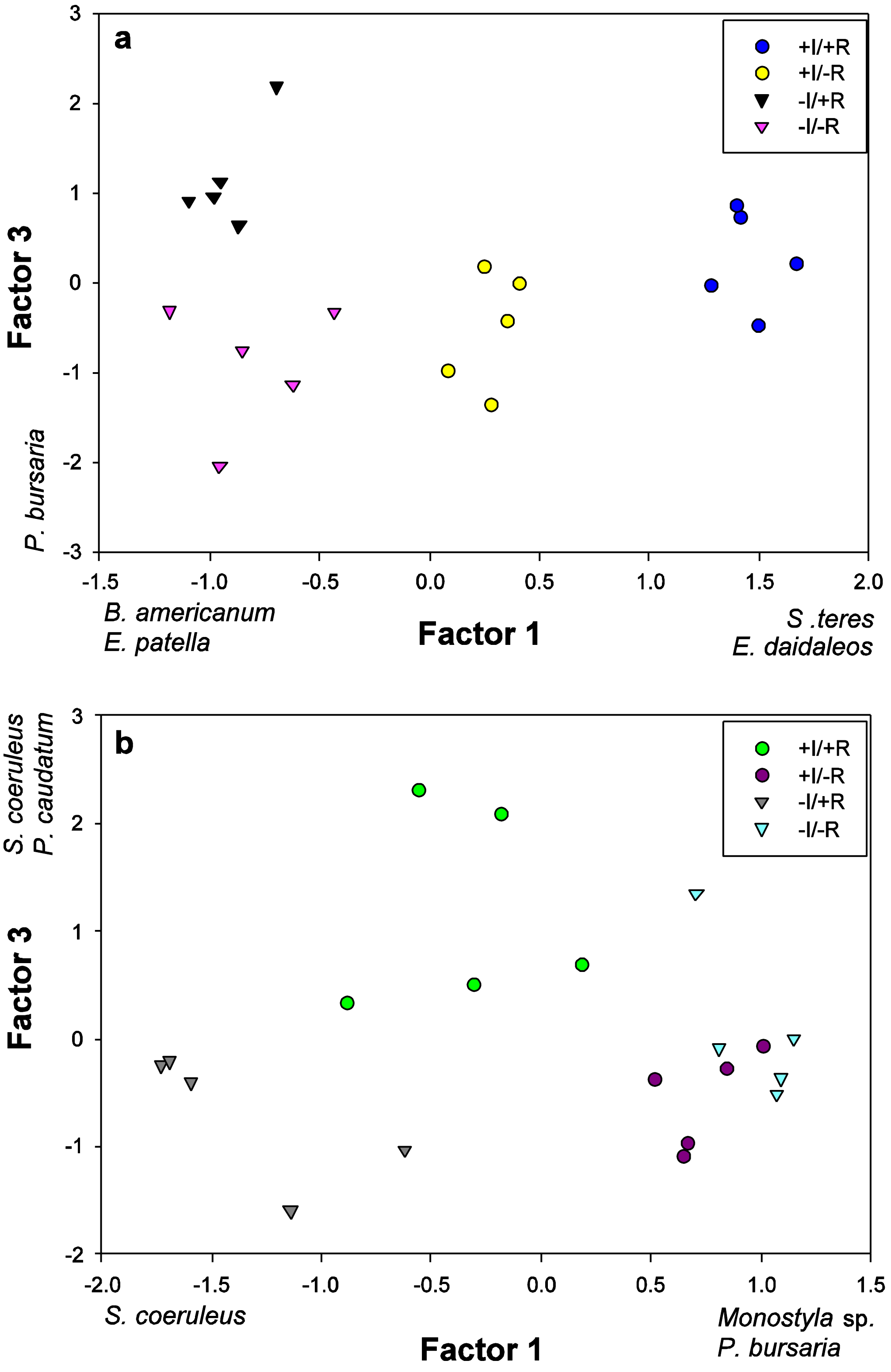

a
S. coeruleus log abundance (per mL)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
ab
c
bc
ab
a
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
P. caudatum log abundance (per mL)
1.6
1.7
1.8
1.9
2.0
2.1
2.2
a
ab
ab
ab
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
Monostyla sp. log abundance (per mL)
1.4
1.6
1.8
2.0
2.2
2.4
2.6
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
E. daidaleos log abundance (per mL)
0.8
1.0
1.2
1.4
1.6
1.8
a
ab
ab
a
b
+I/+R
+I/-R
-I/-R
-I/+R
-R

Figure 2.5a,b. Community response to potential evolutionary history of resident and invading species.

Independent or shared potential evolutionary history among the invader (I) and the resident (R) species is denoted by a “-” or “+” respectively in the legend. a, the response of assemblage A for factors 1 and 3 (n = 5). The MANOVA of three principal components that accounted for 78.32% (factor 1), 10.59% (factor 2), and 6.34% (factor 3) of variance, supported a highly significant overall effect of interaction history on composition ($F_{9, 34.2} = 22.95$, $p < 0.0001$). Interaction history between the invader and the native community affected factor 1 ($F_{3,16} = 172.24$, $p < 0.001$) and factor 3 ($F_{3,16} = 16.26$, $p = 0.0004$). b, the response of assemblage B for factors 1 and 3 (n = 5). Factor 1 accounted for 81.49% of the variance, factor 2 for 11.04%, and factor 3 for 4.13%. The overall effect of interaction history on assemblage B’s composition was highly significant ($F_{9,34.2} = 12.05$, $p < 0.0001$). Interaction history affected factors 1 ($F_{3,16} = 49.63$, $p < 0.0001$) and 3 ($F_{3,16} = 7.38$, $p = 0.0025$).


a
● +I/+R
● +I/-R
▼ -I/+R
▼ -I/-R
Factor 3
P. bursaria
3
2
1
0
-1
-2
-3
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
B. americanum
E. patella
Factor 1
S .teres
E. daidaleos
b
● +I/+R
● +I/-R
▼ -I/+R
▼ -I/-R
S. coeruleus
P. caudatum
Factor 3
3
2
1
0
-1
-2
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
S. coeruleus
Factor 1
Monostyla sp.
P. bursaria

CHAPTER 3

Evolution alters post-invasion temporal dynamics in experimental communities

Abstract

The causes and consequences of temporal variation in the abundance of organisms constitute central themes in ecological inquiry. Rapid evolution can occur over ecological time scales, potentially resulting in altered temporal variation in abundance and complicating inferences about the consequences of temporal variation. We used 2-species models to generate predications about the effects of rapid evolution on temporal variation of species' abundances in competitive and predator-prey interactions. We then assessed whether evolution altered the temporal variability in species' abundances by comparing founder populations and their evolved descendants in experimental communities of ciliates and rotifers following invasion by a novel species. Models suggest that 1) populations of species that evolved improved competitive ability will experience increased variability in abundance with increasing population size, while populations of inferior competitors will experience decreased abundance and variability in abundance, and 2) if evolution resulted in higher attack rates, prey would exhibit decreased abundance and increased temporal variability in abundance, while predators would exhibit highest abundance and lowest temporal variability in abundance at an intermediate attack rate. Differing evolutionary histories resulted in significant differences among treatments in abundances and temporal variation in abundances of both resident and invading species. Increased abundance and decreased temporal variation are consistent with evolution resulting in reduced attack rates on one invader, *Euplotes daidaleos*, in the novel community. Increased temporal variability in populations of resident species after biological invasions may predispose those populations to extinction, while decreased temporal variability in invader abundance could facilitate invader persistence. These effects on community dynamics may provide one mechanism by which evolution can exacerbate invasions in some communities and ameliorate invasions in others. Evolutionary history alone can affect temporal variation in the abundances of species, generating important consequences for interspecific interactions among species and complicating inferences about the consequences of temporal variability in biological communities.

Key words: community ecology, eco-evolution, invasion ecology, microevolution, temporal variability

Introduction

The causes and consequences of temporal variation in the abundance of organisms constitute central themes of ecological inquiry. Temporal variation in abundance has served as a surrogate for population stability (Connell & Sousa 1983; Pimm 1991), a predictive correlate of extinction risk (Lande 1993; Inchausti & Halley 2003), and a possible early indicator of regime shifts (Scheffer *et al.* 2009; Pace *et al.* 2013). In all of these approaches, temporal variation in population size is usually assumed to be a consequence of invariant traits of species interacting with variable environments or with other species with equally fixed traits. Rapid evolution, however, can occur over what are commonly termed ‘ecological time scales’ (Chao *et al.* 1977; Yoshida *et al.* 2003; Urban 2010; Hiltunen & Becks 2014; Faillace & Morin 2016), resulting in changes in the traits of species that influence their dynamics, including temporal variation in abundance. Consequently, it is of interest to explore whether short-term evolution alters patterns of temporal variation in interacting sets of species, and if so, whether those alterations are in predictable directions or are highly idiosyncratic.

Short-term evolution could influence temporal dynamics in a number of ways. In exploiter-victim interactions, the evolution of increased defences with or without reduced virulence of exploiters can reduce population fluctuations (Chao *et al.* 1977), resulting in decreased temporal variation. In contrast, cyclical selection for resistant or vulnerable strains might lead to fluctuations of different periodicity but similar amplitude, producing no net change in overall temporal variation in long time series (Yoshida et al. 2003). To the extent that selection on species traits also stabilizes population dynamics resulting from interactions with other species, evolution might result in reduced temporal variation. A reasonable null hypothesis is that if evolution tends to produce larger average population sizes over time, an increase in temporal variation might result as a simple statistical artefact of positive correlations between the mean and variance (Williamson 1972).

In the context of biological invasions, evolution that results in altered temporal variability of populations of invading or native species could provide one mechanism that either mitigates or exacerbates invasions, or may herald an impending regime shift to a permanently invaded state. Evolution has the capacity to change the course of biological invasions by influencing invader success and native species' responses and modifying the impacts of biological invasions (Carroll 2011) through effects on interspecific interactions (Palkovacs *et al.* 2009; Schoener 2011). Strong evidence for evolution requires measuring traits before and after invaders and residents have interacted for some time (*e.g.*, (Stuart *et al.* 2014), so research aiming to definitively assess how evolution affects long-term invasion dynamics should measure the traits of both resident and invading species early and later in the course of invasions (Kolbe et al. 2012). Because evolution is a dynamic process, we might also expect that initial evolutionary responses might also change over time. Inferences about ongoing evolution are frequently further complicated by an incomplete knowledge of the history of introductions and spread for invading exotic species (Le Roux & Wieczorek 2009; Kolbe *et al.* 2012). These limitations can be addressed by experiments using laboratory organisms with short generation times. The opportunity for potential evolution can be altered by varying the duration of post-invasion interactions (Warren et al. 2006) , while measuring evolutionary outcomes over experimentally tractable time spans.

Here we examine whether temporal variability in abundances differ in invaded communities as a result of evolution. We used 2-species models to generate predications about the effects of evolution on temporal variation of species' abundances in competitive and predator-prey interactions. We then empirically assessed whether evolution altered the temporal variability in species' abundances by comparing founder populations and their evolved descendants in experimental communities of ciliates and rotifers following invasion by a novel species. We used two different species assemblages (hereafter termed A and B) composed of aquatic bacteria, protists and rotifers in which we previously evolution can alter the average abundance of species over time and consequently shift patterns of community structure (Faillace & Morin 2016). By varying whether or not invaders and residents interacted over many generations before assessing performance (*i.e.*, abundance) in a second round of invasion, we determined whether performance of invaders and residents changed over time. Treatments differed in the experienced (*i.e.,* those that had a recent interaction history of approximately 200-400 hundred protist generations) or naïve status (*i.e.*, no recent interaction history) of both invading and resident species. Changes in temporal variation caused by evolution are likely to be complex and will depend on the kinds of dominant interspecific interactions that are affected, competition, predator-prey, etc. Models suggest that 1) populations of species that evolved improved competitive ability will experience increased variability in abundance with increasing population size, while populations of inferior competitors will experience decreased abundance and variability in abundance, and 2) if evolution resulted in higher attack rates, prey will exhibit decreased abundance and increased temporal variability in abundance, while predators will exhibit highest abundance and lowest temporal variability in abundance at an intermediate attack rate.

Methods

Model

We used a standard model of Lotka-Volterra competition equations for two competitors and modified it to incorporate a periodic pulsed dilution environment comparable to our periodic subculturing of our experimental communities every 20 days (Equations 1 and 2) in R (R Core Team 2011) (Appendix 1). Changes in competition coefficients of each competitor (either increased or decreased per capita competitive effects) simulated what might occur as a result of evolutionary changes in competitive ability

$$\frac{dN_1}{dt} = \mathrm{r}_1 N_1\left(1 - \frac{\alpha_{11}N_1 - \alpha_{12}N_2}{K_1}\right) \qquad \text{Equation 1}$$

$$\frac{dN_2}{dt} = \mathrm{r}_2 N_2\left(1 - \frac{\alpha_{22}N_2 - \alpha_{21}N_1}{K_2}\right) \qquad \text{Equation 2}$$

where r_i is the intrinsic rate of increase, N_i is the population size, K_i is the carrying capacity, and α_{ij} is the competition coefficient for each species. To assess the effects of evolution on temporal variability we compared abundances and variability in abundances for both competitors after 400 runs of the model that differed in competitive ability of one or both competitors. We picked parameter values that would yield a stable coexistence of both species, and used a periodic reduction in densities to introduce a source of environmental variation comparable to that produced by our regular subculturing of our experimental communities. See below.

We modified standard Lotka-Volterra predator-prey equations in R (R Core Team 2011) to include a periodic pulsed dilution environment in which we changed the predator attack rate to model possible effects resulting from evolutionary changes in predator ability or prey defence (Equations 3 and 4)

$$\frac{dH}{dt} = bH\left(1 - \frac{H}{K}\right) - aPH \qquad \text{Equation 3}$$

$$\frac{dP}{dt} = eaPH - sP \qquad \text{Equation 4}$$

where *H* is the prey population size, *P* is the predator population size, *b* is the prey rate of increase, *a* is the predator attack rate, *e* is the conversion efficiency of prey into predators, *s* is the predator death rate, and *K* is the prey carrying capacity (Appendix 1). We assessed the effects of evolution on temporal variability by comparing abundances and temporal variability in abundances of both predator and prey among runs of the model that differed in attack rate of the predator.

In both models, we mimicked a periodic dilution event by reducing population sizes to 0.1 of their value on the previous day after growth intervals of 20 days. This introduced a periodic source of temporal variation to what would otherwise be the temporally invariant densities reached at equilibrium.

Experimental Design

We describe the detailed experimental methods elsewhere (Faillace & Morin 2016). To measure the evolutionary responses to different selective regimes imposed by different invasion scenarios, our experimental protocol focused on protists and rotifers with short generation times to facilitate the measurement of evolutionary responses to different invasion regimes. The experiment proceeded in three phases to ensure that populations had ample time for evolution to occur and be measured. Phase one ensured that the resident species (analogous to native species with a shared history of interaction and potential coadaptation) grown in each assemblage, A and B, had sufficient time to interact and potentially coadapt to one another, thus sharing a recent history of interaction that spanned roughly 300-550 protist generations. Phase two invaded replicates of each assemblage a species drawn from the other assemblage, such that the invaders and species in invaded assemblages did not share a recent evolutionary history. Species in the invaded communities then interacted for a sufficient time (approximately 200-400 generations) so that the invaders and residents could potentially evolve. Phase three compared the performance of different lines of potentially evolved invaders and residents to naïve invaders and residents in all possible combinations to assess the effects of recent interaction history on species performance and community composition (see Fig. 1 in (Faillace & Morin 2016). Species composition and external environmental factors remained constant, such that the treatments within each community differed solely in the duration of interaction history. We have explained elsewhere that phenotypic plasticity is an unlikely explanation for the observed differences in response to the same suite of species (Faillace & Morin 2016).

Assemblage A contained five ciliates *Blepharisma americanum, Spirostomum teres, Euplotes patella, Prorodon niveus, Paramecium bursaria,* and a rotifer, *Lecane* sp. (Fig 1a). Assemblage B contained three ciliates *Stentor coeruleus*, *Paramecium caudatum*, *Euplotes daidaleos*, and a rotifer *Monostyla* sp. (Fig 1b). We selected these assemblages for their stability in species composition and had maintained them in laboratory cultures for three years before the start of the experiment (equal to at least 550 to 1095 protist generations depending on species and approximately 275 generations for rotifers). The same four bacterial species, *Proteus vulgaris, Serratia marcesens, Bacillus subtilis,* and *Bacillus cereus*, served as edible resources for the bacterivores. We did not quantify the number and abundances of bacterial species, however, and others were likely introduced along with our focal eukaryotes. Five treatments (with n = 5 microcosms for each) manipulated the duration of interaction history following invasion and possible evolution for assemblage A and B, for a total of 10 treatments across both assemblages.

One species from each assemblage was designated as an experimental invader of the other assemblage. Over a three-week period, we experimentally invaded 5 replicates of resident community A with 15-20 cells of *E. daidaleos* originally grown in source community B, and 5 replicates of resident community B with 15-20 cells of *P. bursaria* originally grown in source community A to create the final treatments used to evaluate performance of each species. The designated invaders were functionally similar (mixotrophic bacteriovores containing symbiotic chlorellae) and the resident assemblages that they invaded contained congeners (*Euplotes patella* and *Paramecium caudatum*) with which they might be expected to interact strongly. The final treatments contained: 1) invading and resident species both experienced (+I/+R), 2) experienced invaders and naïve residents (+I/-R), 3) invaders and residents both naïve (-I/-R), 4) naïve invaders and experienced residents (-I/+R), and 5) an uninvaded control containing only resident species originally present before invasions (-R). We estimated the abundances of all species weekly for twelve weeks.

Statistical analyses

To quantify the effect of evolutionary history on the temporal dynamics of the invaders and resident species, we first calculated species densities as number +1 per mL and then $\log_{10}$-transformed the values before analysis. We then averaged $\log_{10}$-transformed densities within each replicate for the first six-week time period and for the second six-week period to observe both the initial and later post-invasion dynamics for each replicate. We analyzed the variability in species abundances over time by using the standard deviation of log-transformed mean abundance of each species as the response variable. The log transformation is an effective way of decoupling the variance and mean, to eliminate possible changes in temporal variation simply resulting from increases or decreases in average abundance (Bisgaard & Kulahci 2011). For each species in each assemblage, A and B, a Multivariate Analysis of Variance [MANOVA, SAS 9.4 (SAS Institute 2011)] evaluated the effect of evolution treatments on the standard deviation of log-abundance initially (first six weeks after final round of invasions) and a separate analysis evaluated long-term effects (second six weeks after final round of invasions). By examining the effects of treatments in two consecutive time periods (Fig. 2) we can observe differences that closely reflect the recent evolutionary history of the populations in the initial observation period, as well as potentially observe populations that were initially evolutionarily naïve begin to change as evolution progresses in the later observation period.

In assemblage A, difficulty in locating *P. niveus* individuals in the experienced invader/experienced residents (+I/+R) treatment resulted in small initial population size when we established the –I/+R treatment. *P. niveus* subsequently failed to become established in the –I/+R treatment, likely because of this exceptionally small initial population size. For this resident species and each invader, we instead conducted separate Analyses of Variance (ANOVAs) for temporal dynamics. We evaluated significant pairwise differences between treatment means using Tukey's Honestly Significant Difference (HSD) multiple comparison test (at $\alpha = 0.05$) to help identify which treatments differed in abundance. We also examined the correlation between the mean and standard deviation for each species to ensure that any differences in variability we observed were not due simply to statistically significant positive correlations between the mean and variance.

Results

Models

In both models, the pulsed dilution environment resulted in forced cycling of abundances of species, roughly comparable to temporal dynamics in abundance observed empirically in our experimental conditions. When competitive ability (α_{12}) was changed between runs of the model to simulate an evolutionary change in one parameter while holding all other parameters constant, increased competitive ability in species 1 (*i.e.* the superior competitor) slightly increased its mean abundance and temporal variability of abundance, while more strongly reducing the abundance and variability in abundance of the inferior competitor, species 2, over time (Table 1, Fig. 3). For the predator-prey model, increasing predator attack rate (*a*) decreased prey abundance while increasing the temporal variability in prey abundance. The effect of increasing *a* on the predator was variable with the highest abundance and lowest variability in abundance occurring at an intermediate attack rate (Table 2, Fig. 4). The changes in abundance and temporal variability were larger for the prey species than for the predator. These results provide some insights about how evolutionary changes in competitive ability or attack rates might affect the mean and variance of abundance over time.

Temporal variability in species' abundances

Patterns of mean abundance during the early period have been previously reported in Faillace and Morin (2016). Patterns of mean abundance for the late period are reported for the first time here. Evolution increased the mean abundance and altered temporal dynamics for one invader, *E. daidaleos* (early: $F_{3, 16} = 3.57$, p = 0.0387; late: $F_{3. 16} = 4.53$, p = 0.0175), but did not alter temporal dynamics in abundance for *P. bursaria* (early: $F_{3, 16} = 0.98$, p = 0.4283; late: $F_{3, 16} = 0.74$, p = 0.5419) (Fig. 5). Evolution reduced the temporal variability in the abundance of *E. daidaleos* in treatments with evolved invaders (+I) relative to treatments with naïve invaders (-I) (Fig. 5a). In the late period temporal variability in the naïve invader/evolved residents (-I/+R) treatment remained significantly higher than either treatment with evolved invaders (+I) (Fig. 5c). Temporal variance in abundance was negatively correlated with abundance for *E. daidaleos* (early: r = -0.67728, p = 0.0010; late: r = -0.65390, p = 0.0018, Appendix 2) but uncorrelated for *P. bursaria*. Initially, evolutionary history affected the temporal dynamics for some residents in both communities (Figs 6 and 7), but this effect was only significant for assemblage A in the late observation period (assemblage A, early: $F_{20, 54.016} = 3.16$, p = 0.0004, late: $F_{20, 54.016} = 2.38$, p= 0.0060; assemblage B early: $F_{16, 52.573} = 1.93$, p = 0.0382, late: $F_{16, 52.573} = 1.29$, p = 0.2399) (Figs 8 and 9).

For assemblage A, invasion by *E. daidaleos* resulted in greater temporal variability for four resident species relative to the uninvaded state (Figs 6 and 8). Differences varied idiosyncratically among species, however most differences among treatments were largest in the early observation period. Variability seemed to be greatest in treatments containing the evolved invader (Fig. 6). For most species displaying significant differences in temporal variability among treatments, mean abundance and temporal variation in mean abundance were either negatively correlated or uncorrelated (Appendix 2). Only for *E. patella* and *P. niveus*, in which abundance dropped below detection threshold (Faillace & Morin 2016), were abundance and temporal variation positively correlated. For *E. patella*, initial mean abundance and temporal variability were negatively correlated and invasion caused greater temporal variation (Fig. 6c). Abundance in the late observation period declined so precipitously in treatments with the evolved invader (+I) (Faillace & Morin 2016), that variability in abundance then declined as well (Fig. 7c). Positive correlation between abundance and temporal variability in abundance of *P. niveus* at both time periods suggests that *P. niveus* abundance was so low that we were unable to reliably track variability in its abundance (Figs 6e and 7e). Temporal variability in two species in assemblage B, *S. coeruleus* and *Monostyla* sp., differed initially among treatments (Fig. 7a,d), with highest variability in the naive invader/evolved residents (-I/+R) treatment, however differences did not persist long-term (Fig. 9).

Discussion

Evolutionary impacts on temporal variability complicate inferences about the consequences of temporal variation. An important assumption of theory and work tying temporal variability to stability, extinction risk, and regime shifts is that observed temporal variability results from unvarying traits of species interacting with other species or the environment rather than ongoing evolution that alters those traits. Evolution can occur rapidly, at ecologically meaningful timescales (*e.g.* see Urban 2010; Hiltunen & Becks 2014) suggesting that evolutionary change in traits could be an important underlying cause of temporal variability in species' abundances. Here we have shown that evolutionary history alone can affect temporal variation in abundance, generating important consequences for interspecific interactions among species and complicating inferences about the consequences of temporal variability in biological communities.

For *E. daidaleos*, the empirical results from our experiment appear to follow a pattern similar to those suggested by our predator-prey model. In Faillace and Morin (2016), we showed that evolution of *E. daidaleos*, caused the observed differences in species abundances among treatments, such that treatments with the evolved invader were most similar to one another. Here we show that *E. daidaleos* exhibited significantly decreased temporal variability concurrent with increased abundance, suggesting that it may have evolved increased predator defence. Two species in assemblage A, *P. niveus* and *B. americanum* are both potential predators able to consume *E. daidaleos*. Our previous results also showed that abundance of *B. americanum* decreased in treatments with ongoing evolution. Temporal variability in the abundance of *B. americanum* increased in the coevolved treatment (+I/+R) relative to the uninvaded control (–R), but the variability in abundance in the remaining treatments was intermediate. Because *B. americanum* is an intraguild predator, its dynamics may be particularly complex and difficult to predict with simple two species models like the ones we used here. Possible effects on the temporal variability of *P. niveus* are difficult to evaluate because abundance and temporal variability were positively correlated during our experiment. It is clear, however, that performance of *P. niveus* declined precipitously over the course of invasion, an outcome that could plausibly result from decreasing availability of suitable prey as *E. daidaleos* evolved to become less edible and additional prey species, like *E. patella*, simultaneously declined dramatically in abundance.

For both *P. niveus* and *E. patella*, a resident congener to *E. daidaleos*, we observed that that these species became functionally extinct within communities where species had extensive opportunities to evolve. The present study suggests a possible mechanism for these extinctions. Evolution of the invader, *E. daidaleos*, may have decreased the temporal variability in its own performance while concurrently greatly reducing the abundances of *E. patella* and *P. niveus* and leading to increased temporal variability in abundance of *E. patella*. By simultaneously reducing abundance and increasing temporal variability in abundance, evolution of the invader created conditions that increased the risk of extinction for these residents through purely stochastic mechanisms, while also facilitating its invasion by reducing its own susceptibility to stochastic events.

Eco-evolutionary dynamics in biological invasions are emerging as an important area of study in invasion ecology. Earlier research has demonstrated that both invading and native species can exhibit evolved trait changes during the course of biological invasions (*e.g.*, Lee 2002; Strauss *et al.* 2006a; Carroll 2007a; Carroll 2007b; Mealor & Hild 2007; Phillips *et al.* 2010; Goergen *et al.* 2011; Shine 2012; Strauss 2012; Colautti & Lau 2015), but it has remained unclear what consequences such evolution has at the community level. In natural systems, long-term observations suggest that interactions among native and invading species can change as a result of evolution (Carlsson *et al.* 2010; Carthey & Banks 2015). Recent experimental evidence indicates that ongoing evolution as a result of biological invasions has the ability to alter the consequences of biological invasions by changing interspecific interactions and community structure (Faillace & Morin 2016), and that evolutionary history can result in altered ecosystem properties (Palkovacs et al. 2009). Our current work demonstrates that evolution following biological invasions also affects temporal variability in abundances of species. Increased temporal variability in populations of resident species after biological invasions may predispose those populations to extinction, while decreased temporal variability in invader abundance could facilitate invader persistence. These effects on community dynamics may provide one mechanism to explain how evolution can exacerbate invasions in some communities and ameliorate invasions in others.

Eradication of invaders is infeasible for the vast majority of exotic species (Lockwood et al. 2013). Evolution of traits resulting from novel selective pressures during biological invasions can significantly alter species interactions, community properties, and ecosystem functioning, making it insufficient to solely measure the traits that may be under selection during the invasion process. The growing interest in eco-evolutionary dynamics of invasions will continue provide important insight into how ongoing evolution will alter the consequences to community dynamics in invaded systems during the course of biological invasions.

Table 3.1. Model output of mean of log abundance and the standard deviation (SD) of log abundance for two competitors in a 2-species Lotka-Volterra model when evolution alters the competitive ability (α) of one competitor.

In all model runs initial densities and all other parameters were held constant. The intrinsic rate of increase for both species was set to 0.2 ($r_1 = r_2 = 0.2$) and carrying capacity for both species = 1000 ($K_1 = K_2 = 1000$).

Competition coefficients		**Competitor 1**		**Competitor 2**	
α_{12}	**α_{21}**	**Mean of log abundance**	**SD of log abundance**	**Mean of log abundance**	**SD of log abundance**
0.2	0.6	2.0489	0.4741	2.2853	0.5404
0.35	0.6	2.0841	0.4837	2.2470	0.5301
0.5	0.6	2.1225	0.4948	2.1956	0.5159
0.5	0.75	2.0144	0.4665	2.2290	0.5264
0.5	0.9	1.8369	0.4345	2.2617	0.5372

Table 3.2. Model output of mean of log abundance and the standard deviation (SD) of log abundance for predator and prey species in a 2-species Lotka-Volterra model when evolution alters the attack rate of the predator.

In all model runs initial densities and all other parameters were held constant. The rate of increase for the prey was set to 1.5 (*b* = 1.5) and carrying capacity = 100 (K = 100). Initial density of prey = 25. Conversion efficiency of prey to predator biomass = 0.1 (*e* = 0.1), predator death rate = 0.5 (*s* = 0.5). Initial density of predators = 15.

	Prey (H)		**Predator (P)**	
Attack rate (*a*)	**Mean of log abundance**	**SD of log abundance**	**Mean of log abundance**	**SD of log abundance**
0.1	1.6948	0.3100	0.2054	0.5449
0.2	1.3470	0.3648	0.2624	0.5136
0.3	1.0650	0.5374	0.1195	0.5434

Figure 3.1a, Species in assemblage A, and b, species in assemblage B.

Light grey boxes denote bacterial resource species, medium grey boxes and black boxes (primary consumers and secondary consumers respectively) denote native heterotrophic ciliates and rotifers, and dashed grey boxes indicated invaders. A solid black line indicates an observed interaction, while a dotted black line indicates a possible interaction. New interactions involving the invader are indicated with a dashed and dotted grey line. Adapted from Faillace and Morin (2016).

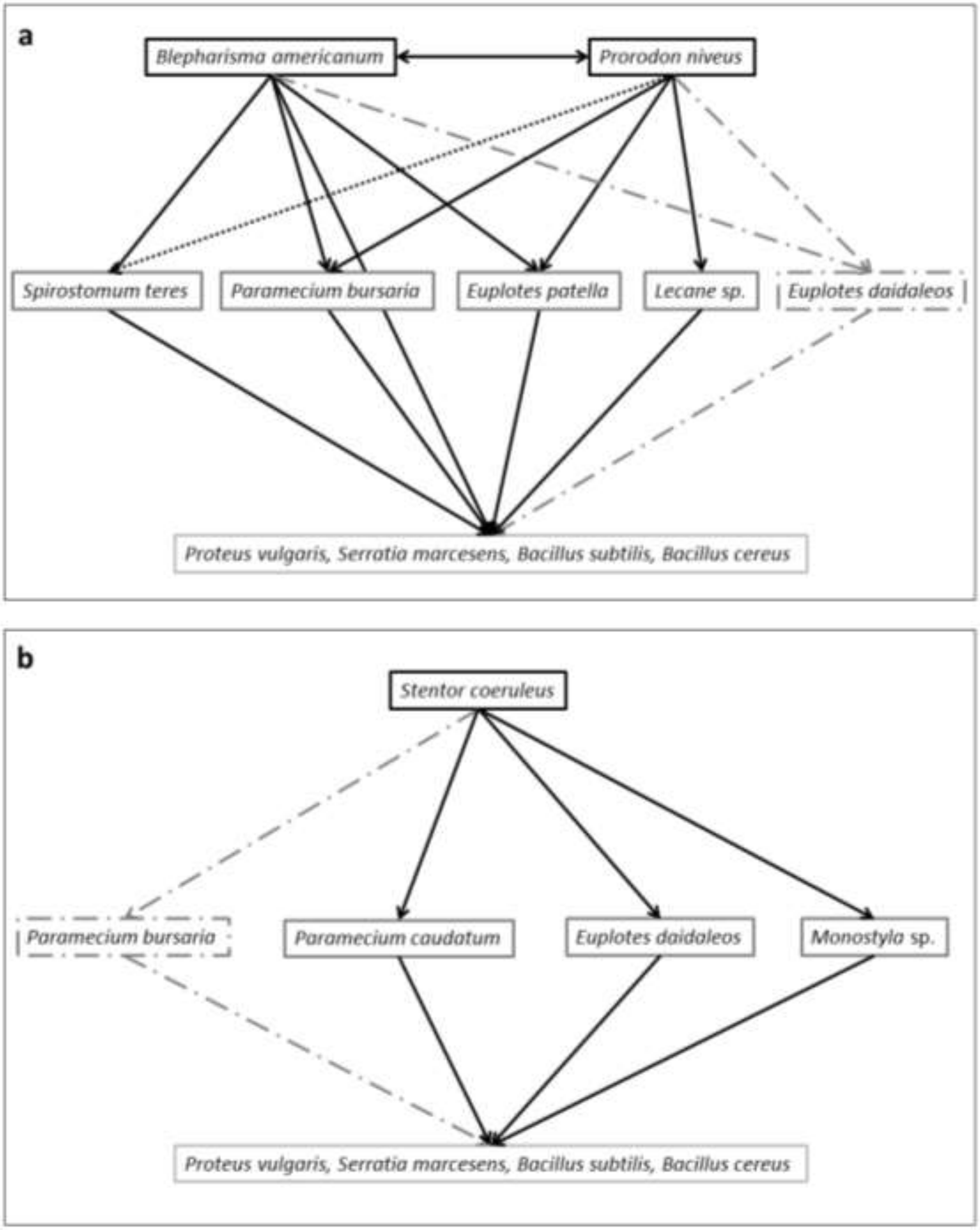

a
Blepharisma americanum
Prorodon niveus
Spirostomum teres
Paramecium bursaria
Euplotes patella
Lecane sp.
Euplotes daidaleos
Proteus vulgaris, Serratia marcesens, Bacillus subtilis, Bacillus cereus
b
Stentor coeruleus
Paramecium bursaria
Paramecium caudatum
Euplotes daidaleos
Monostyla sp.
Proteus vulgaris, Serratia marcesens, Bacillus subtilis, Bacillus cereus

Figure 3.2. Example of log-transformed abundance data showing temporal variability in abundances of species for one replicate community of assemblage A. This example is a replicate from the evolved invader/naïve residents (+I/-R) treatment. The initiation of Phase 3 (starting with the second round of invasions), and the periods of early and late post-invasion dynamics are noted.

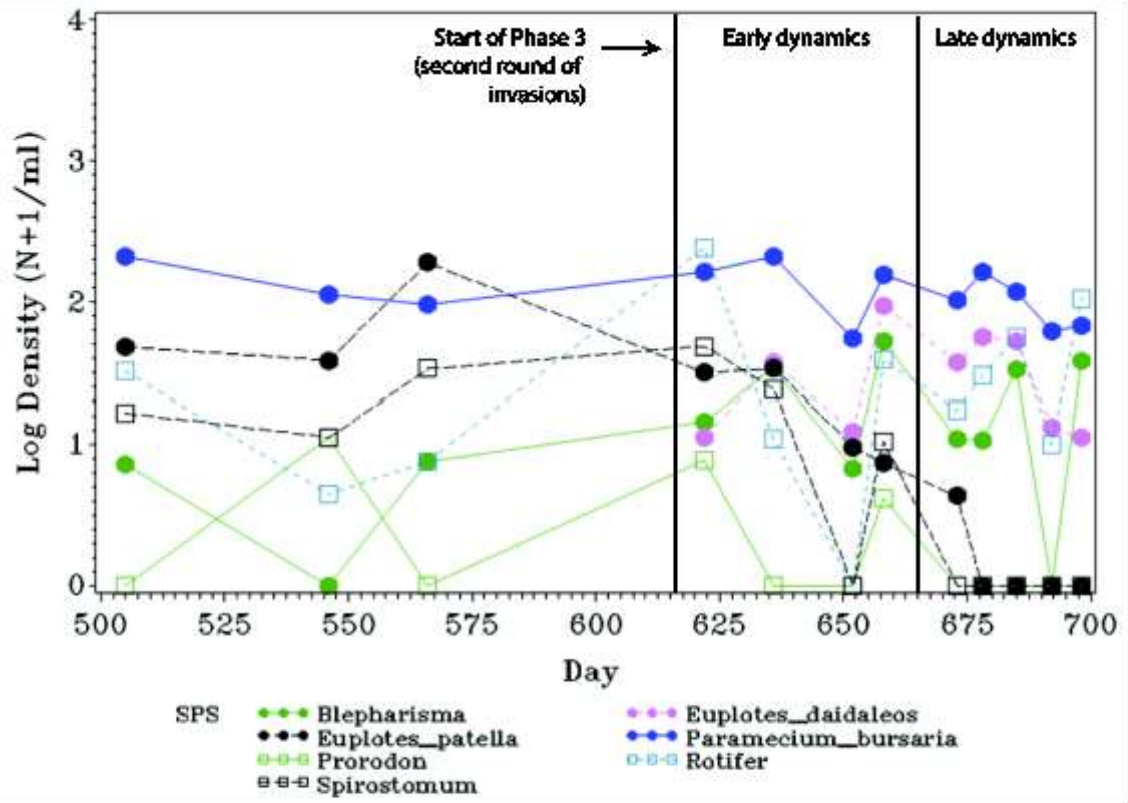


Figure 3a-d. Output from a two species Lotka-Volterra competition model in which the competition coefficient α of one species varied.

In **a,** $\alpha_{12} = 0.2$, $\alpha_{21} = 0.6$, in **b,** $\alpha_{12} = 0.35$, $\alpha_{21} = 0.6$, in **c,** $\alpha_{12} = 0.5$, $\alpha_{21} = 0.6$, and in **d,** $\alpha_{12} = 0.5$, $\alpha_{21} = 0.75$. In all model runs initial densities and all other parameters were held constant. The intrinsic rate of increase for both species was set to 0.2 ($r_1 = r_2 = 0.2$) and carrying capacity for both species = 1000 ($K_1 = K_2 = 1000$).

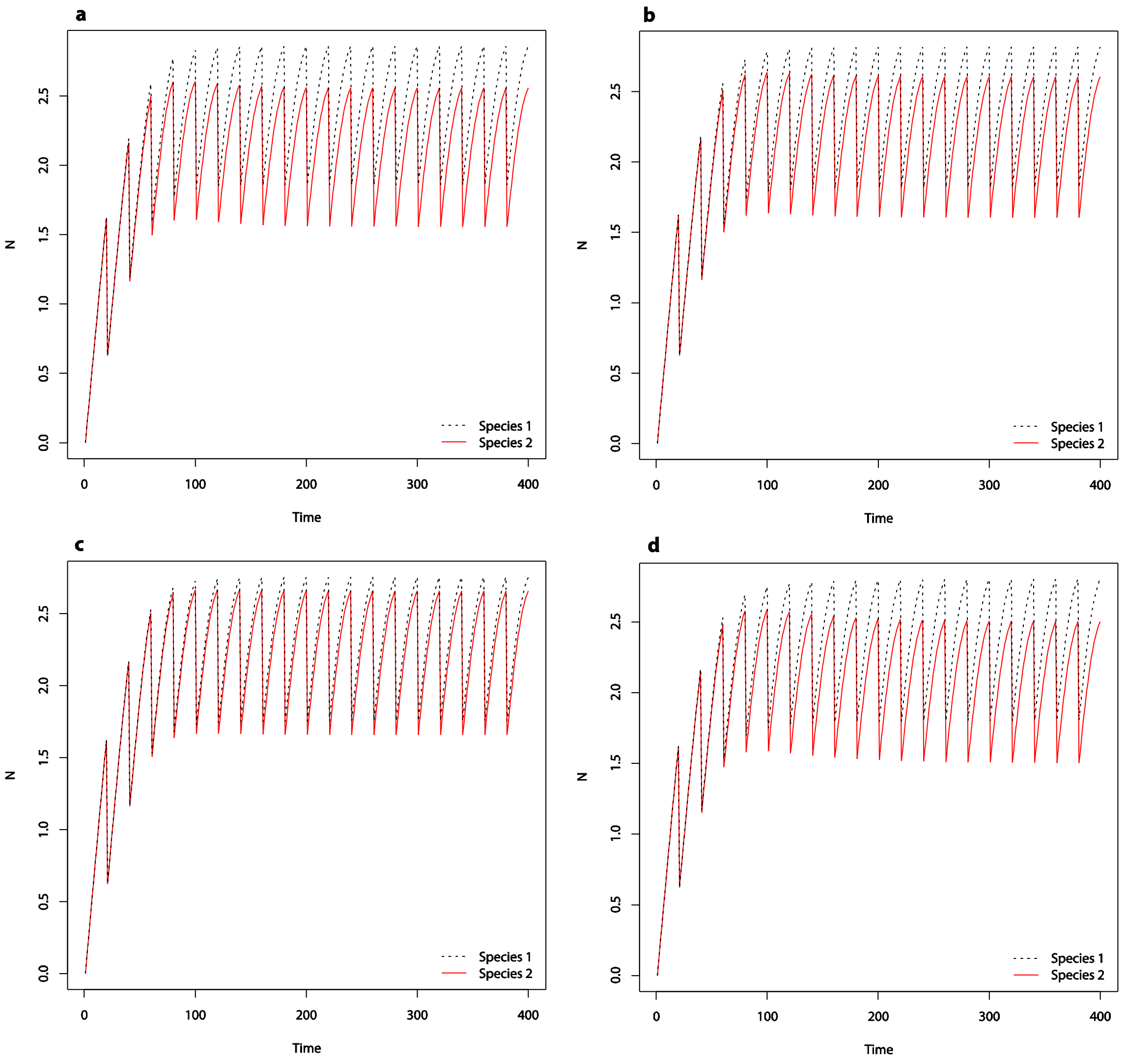
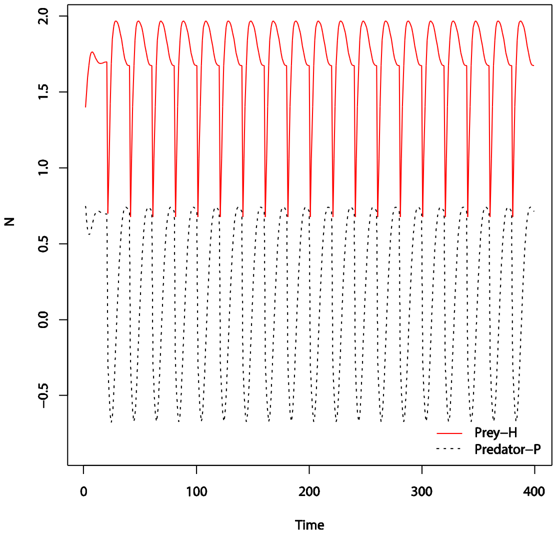


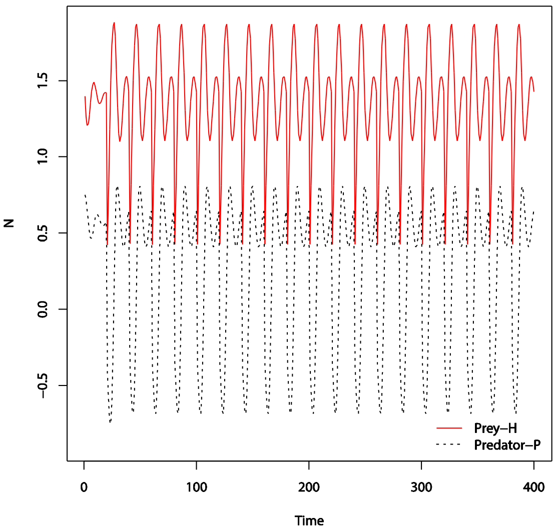
Figure 3.4a-c. Output from a two species Lotka-Volterra predator-prey in which the attack rate (*a*) of the predator varied.

In **a,** *a* = 0.1, in **b,** *a* = 0.2, and in **c,** *a* = 0.3. In all model runs initial densities and all other parameters were held constant. The rate of increase for the prey was set to 1.5 (*b* = 1.5) and carrying capacity = 100 (K = 100). Initial density of prey = 25. Conversion efficiency of prey to predator biomass = 0.1 (*e* = 0.1), predator death rate = 0.5 (*s* = 0.5). Initial density of predators = 15.

a


N
2.0
1.5
1.0
0.5
0.0
−0.5
0
100
200
300
400
Time
Prey−H
Predator−P

b


N
1.5
1.0
0.5
0.0
−0.5
0
100
200
300
400
Time
Prey−H
Predator−P

c

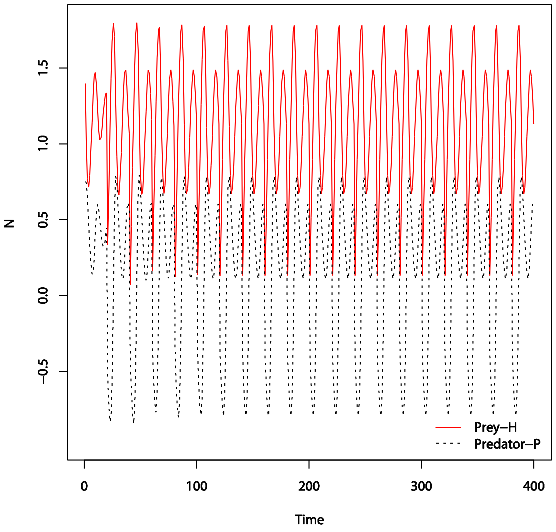

N
1.5
1.0
0.5
0.0
−0.5
0
100
200
300
400
Time
Prey−H
Predator−P

Figure 3.5a-d. Response in temporal variability as a result of evolutionary history by both invaders.

a, early, and **c,** late observed responses for *E. daidaleos* (n = 5) and **b**, early, and **d,** late responses for *P. bursaria* (n = 5). Treatment groups are labelled on the x-axis with a "+" or "-" for experienced or naïve respectively, while an "I" or "R" refers to the invader or species in the resident community, respectively, while "S" refers to species from the source community. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.

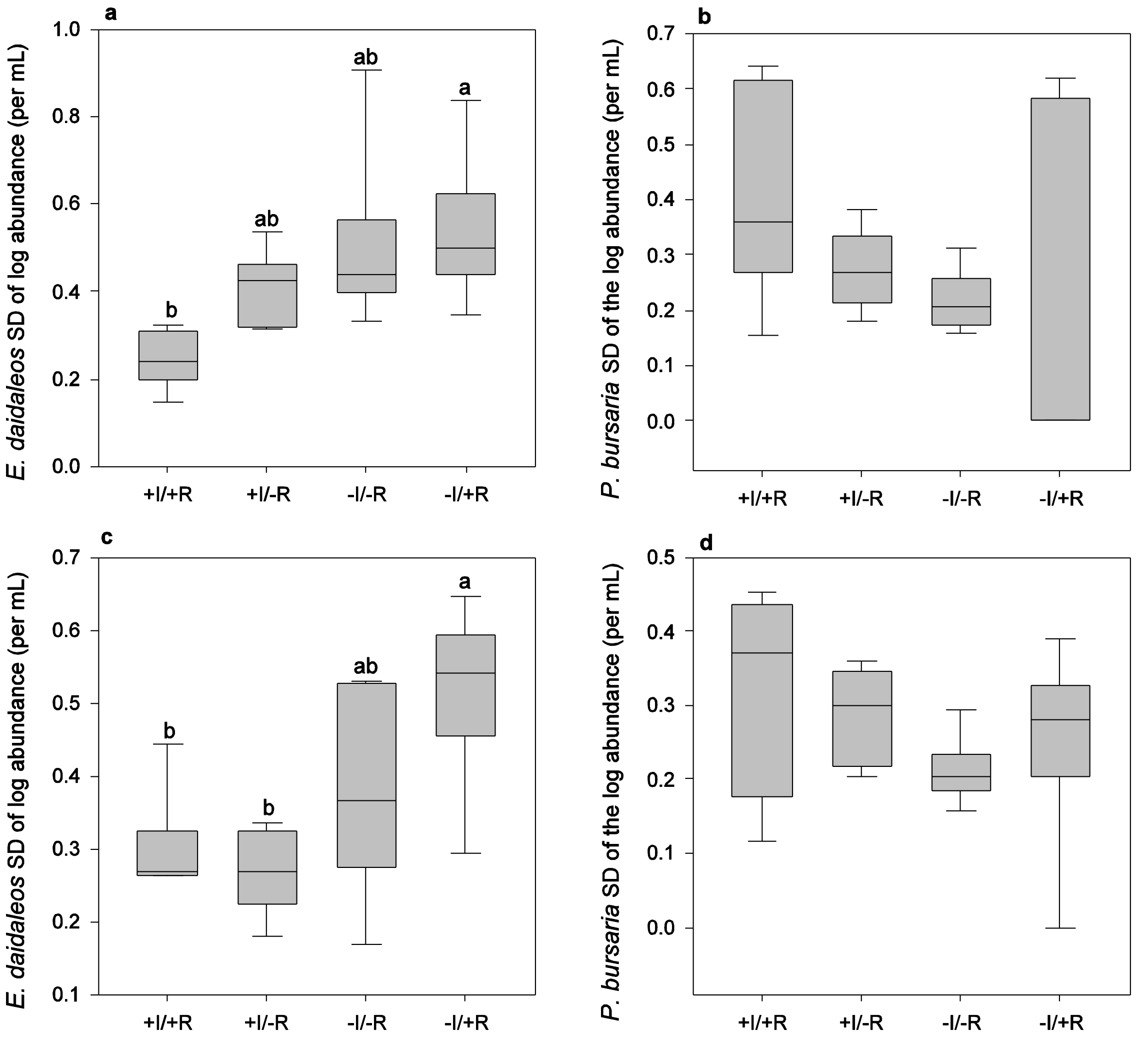

a
E. daidaleos SD of log abundance (per mL)
1.0
0.8
0.6
0.4
0.2
0.0
b
ab
ab
a
+I/+R
+I/-R
-I/-R
-I/+R
b
P. bursaria SD of the log abundance (per mL)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
+I/+R
+I/-R
-I/-R
-I/+R
c
E. daidaleos SD of log abundance (per mL)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
b
b
ab
a
+I/+R
+I/-R
-I/-R
-I/+R
d
P. bursaria SD of the log abundance (per mL)
0.5
0.4
0.3
0.2
0.1
0.0
+I/+R
+I/-R
-I/-R
-I/+R

Figure 3.6a-f. Early response in temporal variability for resident species of assemblage A to evolutionary history with the invader, *E. daidaleos* (n = 5). Treatment groups are labelled on the x-axis with a "+" or "-" for evolved or naïve respectively, while an "I" or "R" refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.

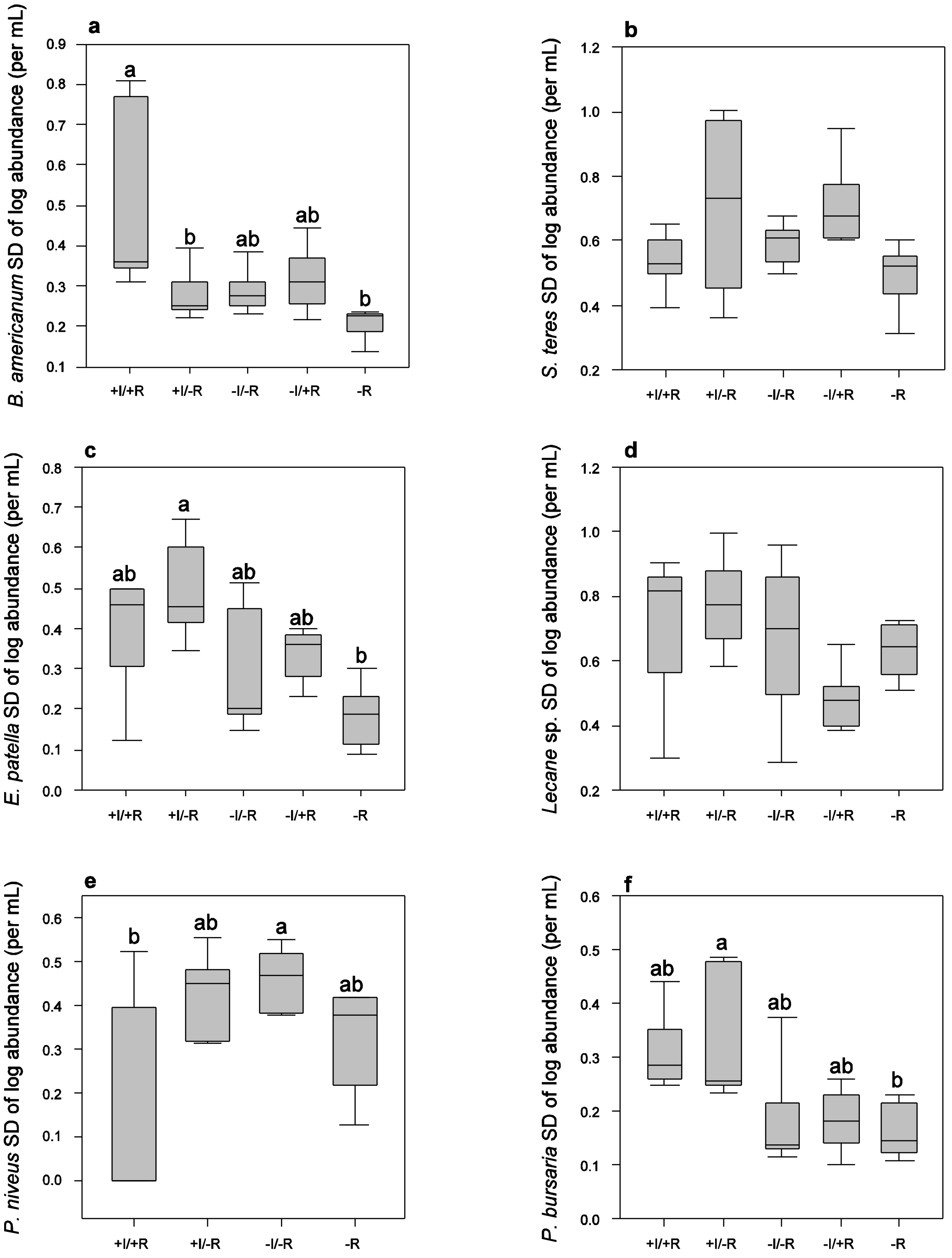

a
B. americanum SD of log abundance (per mL)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
a
b
ab
ab
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
S. teres SD of log abundance (per mL)
0.2
0.4
0.6
0.8
1.0
1.2
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
E. patella SD of log abundance (per mL)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
ab
a
ab
ab
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Lecane sp. SD of log abundance (per mL)
0.2
0.4
0.6
0.8
1.0
1.2
+I/+R
+I/-R
-I/-R
-I/+R
-R
e
P. niveus SD of log abundance (per mL)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
b
ab
a
ab
+I/+R
+I/-R
-I/-R
-R
f
P. bursaria SD of log abundance (per mL)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
ab
a
ab
ab
b
+I/+R
+I/-R
-I/-R
-I/+R
-R

Figure 3.7a-d. Early response in temporal variability for resident species of assemblage B to evolutionary history with the invader, *P. bursaria* (n = 5). Treatment groups are labelled on the x-axis with a "+" or "-" for evolved or naïve respectively, while an "I" or "R" refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.

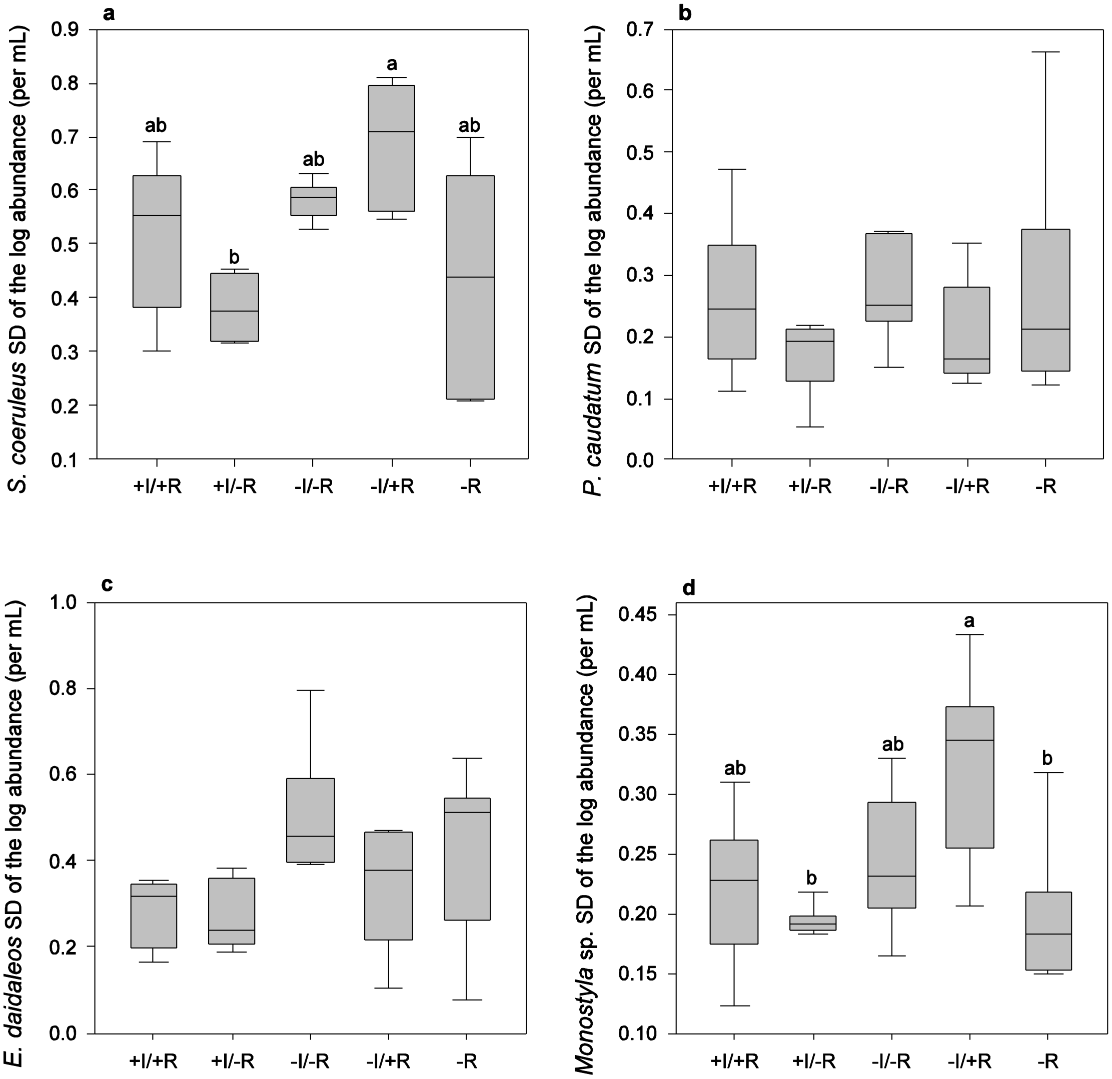

a
S. coeruleus SD of the log abundance (per mL)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
ab
b
ab
a
ab
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
P. caudatum SD of the log abundance (per mL)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
E. daidaleos SD of the log abundance (per mL)
0.0
0.2
0.4
0.6
0.8
1.0
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Monostyla sp. SD of the log abundance (per mL)
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
ab
b
ab
a
b
+I/+R
+I/-R
-I/-R
-I/+R
-R

Figure 3.8a-f. Late response in temporal variability for resident species of assemblage A to evolutionary history with the invader, *E. daidaleos* (n = 5). Treatment groups are labelled on the x-axis with a “+” or “-” for evolved or naïve respectively, while an “I” or “R” refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.

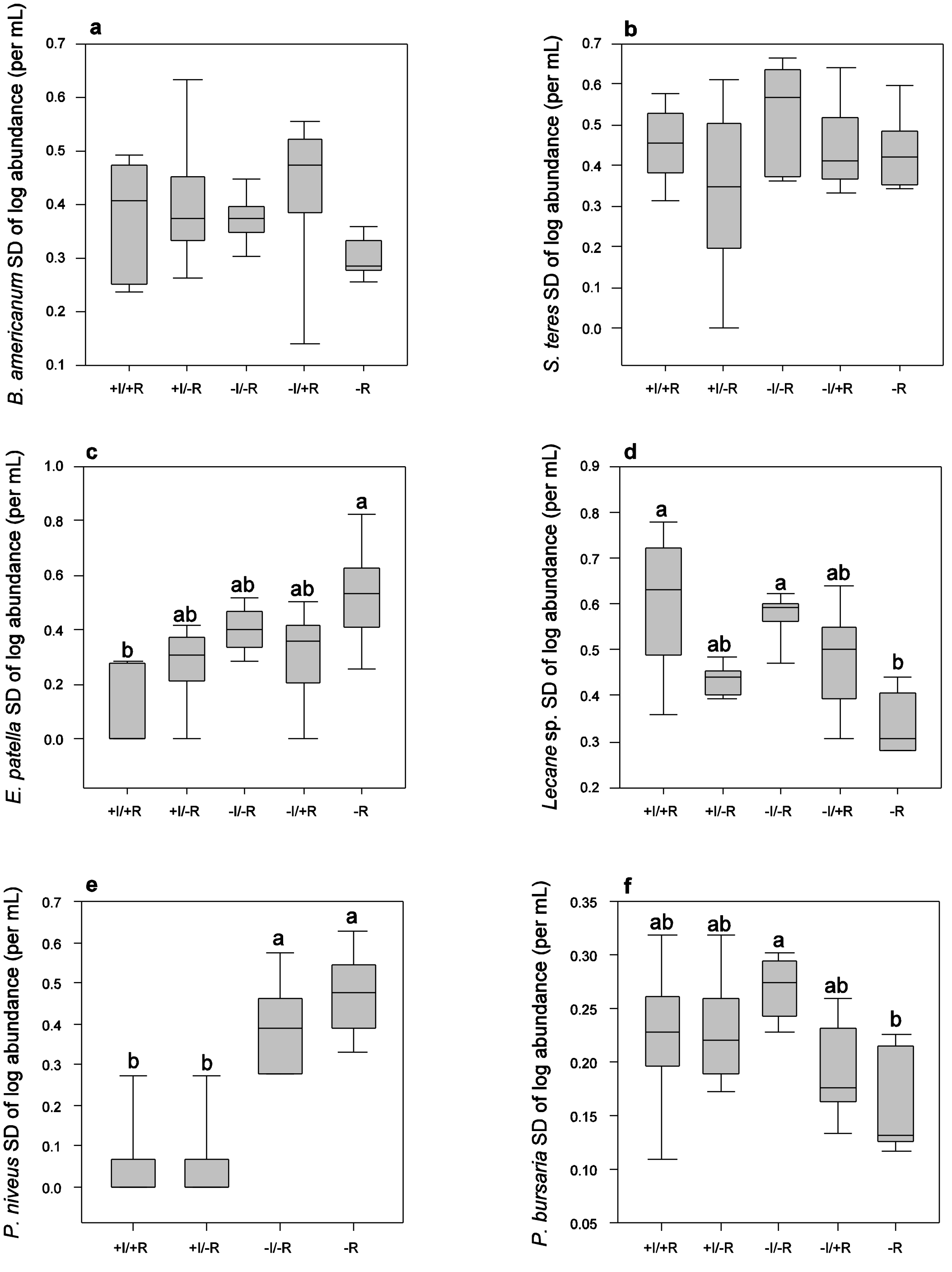
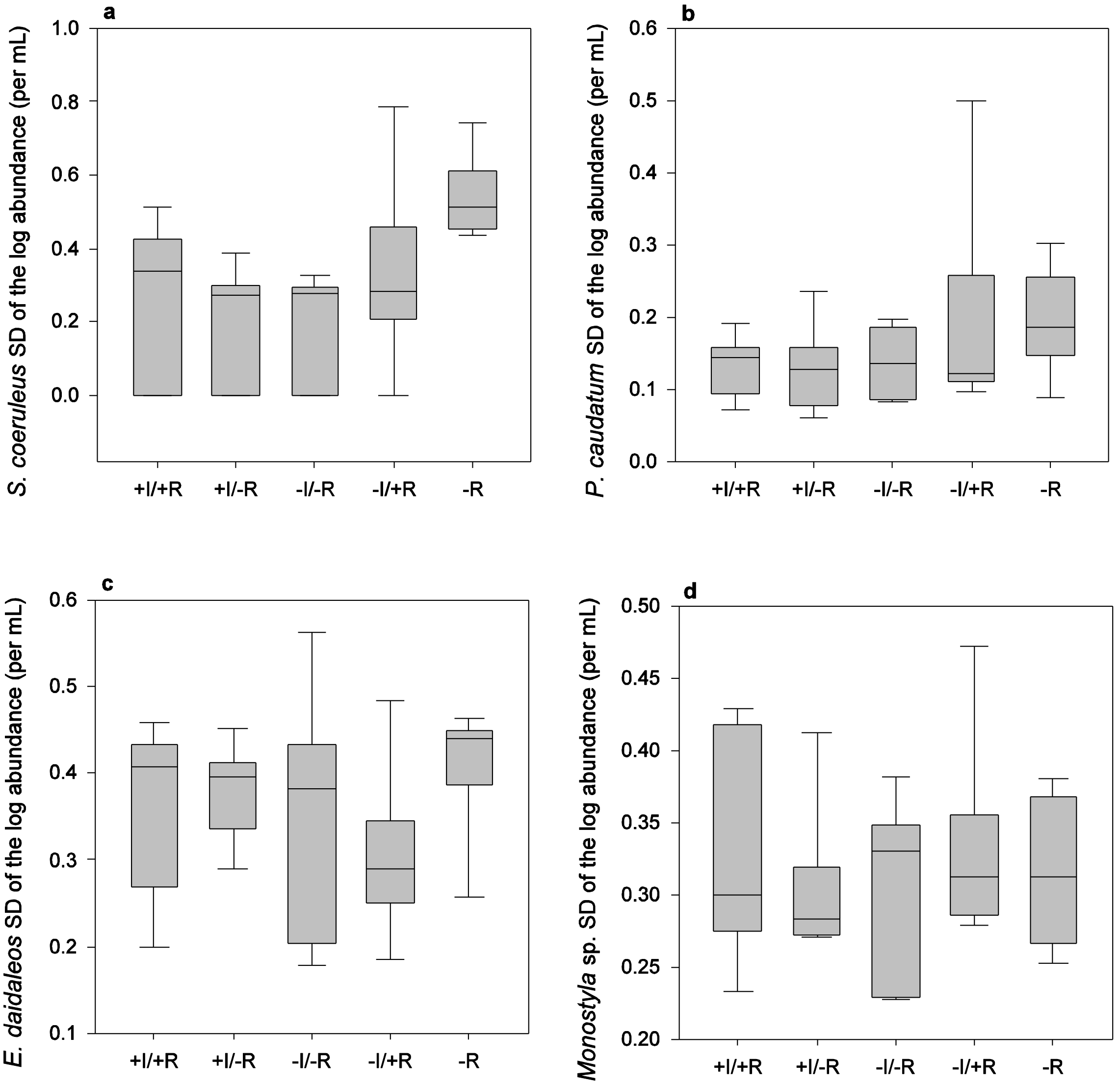

a
B. americanum SD of log abundance (per mL)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
S. teres SD of log abundance (per mL)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
E. patella SD of log abundance (per mL)
0.0
0.2
0.4
0.6
0.8
1.0
b
ab
ab
ab
a
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Lecane sp. SD of log abundance (per mL)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
a
ab
a
ab
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
e
P. niveus SD of log abundance (per mL)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
b
b
a
a
+I/+R
+I/-R
-I/-R
-R
f
P. bursaria SD of log abundance (per mL)
0.05
0.10
0.15
0.20
0.25
0.30
0.35
ab
ab
a
ab
b
+I/+R
+I/-R
-I/-R
-I/+R
-R

Figure 3.9a-d. Late response in temporal variability for resident species of assemblage B to evolutionary history with the invader, *P. bursaria* (n = 5). Treatment groups are labelled on the x-axis with a "+" or "-" for evolved or naïve respectively, while an "I" or "R" refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.


a
S. coeruleus SD of the log abundance (per mL)
1.0
0.8
0.6
0.4
0.2
0.0
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
P. caudatum SD of the log abundance (per mL)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
E. daidaleos SD of the log abundance (per mL)
0.6
0.5
0.4
0.3
0.2
0.1
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Monostyla sp. SD of the log abundance (per mL)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
+I/+R
+I/-R
-I/-R
-I/+R
-R

CHAPTER 4

Evolution reduces ecosystem functioning in invaded communities

Abstract

Evolution can potentially alter the influence of biological invasions on ecosystem functioning. Species traits can evolve in ways that either moderate or exacerbate the impacts of invasions, making it important to consider the effects of evolution in studies of invasions. We explored whether evolution altered the biomass of individual consumer species and the total biomass of protists and rotifers in experimental aquatic communities. Total post-invasion biomass decreased in in two sets of species used for the experiments. In one species assemblage, species that were allowed to evolve displayed decreased biomass relative to the naïve state where little evolution was possible, an effect that persisted through time. In the second assemblage evolution of the resident species initially appeared to restore biomass to pre-invasion levels. This initial effect, however, gradually decayed over time. Ultimately, biomass was significantly lower in all invaded communities relative to controls without invaders, regardless of opportunities for evolution. This suggests that any gains in biomass observed at the species level were more than offset by losses in other species, indicating that evolution at the species level did not result in communities that were more productive over time.

Key words: community ecology, eco-evolution, ecosystem functioning, invasion ecology, microevolution

Introduction

Invasions by exotic species pose a major threat to biodiversity (Simberloff et al. 2013). Though mostly unexplored by experiments (Lambrinos 2004; Strayer *et al.* 2006; Saul & Jeschke 2015), eco-evolutionary dynamics have the capacity to alter the course of biological invasions, with potentially positive and negative effects on the invaded community that modify the impacts of biological invasions (Carroll 2011) through effects on interspecific interactions (Palkovacs *et al.* 2009; Schoener 2011). Evolutionary changes in species during biological invasions can affect community properties (Faillace & Morin 2016), which can in turn alter some aspects of ecosystem functioning.

Biomass is one ecosystem attribute that might be expected to change as a consequence of the evolution of individual species during biological invasions. For individual species, evolution of divergence in resource use by invaders and residents could result in increases in species' biomass if they become better at exploiting complementary resources (*i.e.*, increase niche complementarity, Tilman 1999) . In contrast, increases in competitive ability or defences against predators might result in the increased biomass of some evolved species at the expense of other species (*e.g.,* Chao *et al.* 1977; Rummel & Roughgarden 1983), leading to either no net change in a zero sum scenario, or a net reduction in biomass.

Similar changes in total community biomass may occur. For example, if invaders evolve increased performance and consequently increase in abundance at the expense of native competitor species total biomass production might either be reduced or yield a zero sum result. If, however, invaders increase in performance by evolving to use previously unexploited or complementary resources, biomass production by native competitors may remain unchanged. In such a case, the total community biomass would show an overall increase. When we consider the effects of evolution on more complex communities with higher level consumers, the potential effects on biomass production become even more complicated, making predictions difficult. For instance, if evolution increases invader abundance, it might support a resultant increase in predator abundance, if a resident predator also consumes the invader. Increased predator abundance may in turn result in an increase in apparent competition on resident competitors of the invader, decreasing their biomass. How other species within a food web may compensate for this effect in biomass production becomes murky, making it difficult to predict all of the possible aggregate effects at the community level.

Strong evidence for the effects of post-invasion evolution on species traits requires measuring those traits both before and after invaders and residents have interacted for some time (*e.g.*, Stuart *et al.* 2014). Research aiming to definitively assess how evolution affects long-term invasion dynamics should measure the traits of both resident and invading species early and later in the course of invasions (Kolbe et al. 2012). Because evolution is a dynamic process, we might also expect that initial evolutionary responses might also change over time. Unfortunately, traits of resident and exotic species are often characterized only early in the course of invasions, before much evolution can occur, or only much later, after evolution may have occurred, but when initial measures of trait values are no longer available for comparison. An incomplete knowledge of the history of introductions and spread for invading exotic species frequently further complicates Inferences about ongoing evolution (Le Roux & Wieczorek 2009; Kolbe *et al.* 2012). These limitations can be addressed by experiments using laboratory organisms with short generation times where evolution may occur over tractable time frames. Importantly, the opportunity for evolution to occur can be experimentally altered by varying the duration of post-invasion interactions (Warren et al. 2006), while measuring the consequences of different durations of species interactions for traits of species and properties of communities.

We have shown elsewhere that evolution can alter the average abundance of species invaded communities and consequently shift community composition (Faillace & Morin 2016). Here we examine whether evolution can shift patterns of total biomass production across the community, in an effort to link evolution to one property of ecosystems. We assessed biomass of populations of resident and invading species before and after they had interacted and potentially evolved. We used two different resident species assemblages (hereafter termed A and B) composed of aquatic bacteria, protists and rotifers. By varying whether or not invaders and residents interacted for many generations before assessing performance (*i.e.*, abundance and biomass) in a second round of invasion, we determined whether performance changed over time. Treatments differed in the experienced (*i.e.*, those that had a recent interaction history of approximately 200-400 hundred protist generations) or naïve status (*i.e.*, no recent interaction history) of invading and resident species. Here we address the following general question: Does evolutionary experience of invading or resident species affect total consumer biomass production?

Methods

Experimental Design

We describe the experimental design in complete detail elsewhere (Faillace & Morin 2016). To summarize, we measured the evolutionary responses to different selective regimes imposed by different invasion scenarios, using an experimental protocol focused on protists and rotifers with short generation times to facilitate the measurement of evolutionary responses to different invasion regimes. The experiment proceeded in three phases to ensure that populations had ample time for evolution to occur and be measured. Phase one ensured that the resident species (analogous to native species with a shared history of interaction and potential coadaptation) grown in each assemblage, A and B, had sufficient time to interact and potentially coadapt to one another, thus sharing a recent history of interaction that spanned roughly 300-550 protist generations. Phase two invaded replicates of each assemblage (A or B) with a designated invading species drawn from the other assemblage, such that the invaders and species in invaded assemblages did not share a recent evolutionary history. Species in the invaded communities then interacted for a sufficient time (approximately 200-400 generations) so that the invaders and residents could potentially evolve. Phase three compared the performance of different lines of potentially evolved invaders and residents to naïve invaders and residents in all possible combinations to assess the effects of recent interaction history on species performance and community composition (see Fig. 1 in (Faillace & Morin 2016). Species composition and external environmental factors remained constant, such that the treatments within each community differed solely in the duration of interaction history. We have argued elsewhere that phenotypic plasticity is an unlikely explanation for the observed differences in response to the same suite of species (Faillace & Morin 2016).

Assemblage A contained five ciliates *Blepharisma americanum, Spirostomum teres*, *Euplotes patella, Prorodon niveus*, *Paramecium bursaria*, and a rotifer, *Lecane* sp. Assemblage B contained three ciliates *Stentor coeruleus, Paramecium caudatum, Euplotes daidaleos*, and a rotifer *Monostyla* sp. We selected these assemblages for their stability in species composition and maintained them in laboratory cultures for three years before the start of the experiment (equal to at least 550 to 1095 protist generations depending on species, and approximately 275 generations for rotifers). The same four bacterial species, *Proteus vulgaris, Serratia marcesens*, *Bacillus subtilis,* and *Bacillus cereus*, served as edible resources for the bacterivores. We did not quantify the number and abundances of bacterial species, however, and others were likely introduced along with our focal eukaryotes. Five treatments, including an uninvaded control, (with n = 5 microcosms for each) manipulated the duration of potential evolution / interaction history following invasion and possible evolution for assemblage A and B, for a total of 10 treatments across both assemblages.

One species from each assemblage was designated as an experimental invader of the other assemblage. Over a three-week period, we experimentally invaded 5 replicates of resident community A with 15-20 cells of *E. daidaleos* originally grown in source community B, and 5 replicates of resident community B with 15-20 cells of *P. bursaria* originally grown in source community A to create the final treatments used to evaluate performance of each species. The designated invaders were functionally similar (mixotrophic bacteriovores containing symbiotic chlorellae) and the resident assemblages that they invaded contained congeners (*Euplotes patella* and *Paramecium caudatum*) with which they might be expected to interact strongly. The final treatments contained: 1) invading and resident species both experienced (+I/+R), 2) experienced invaders and naïve residents (+I/-R), 3) invaders and residents both naïve (-I/-R), 4) naïve invaders and experienced residents (-I/+R), and 5) an uninvaded control containing only resident species originally present before invasions (-R). We estimated the abundances of all species weekly for twelve weeks, split *a priori* into two observation periods because we were uncertain how rapidly evolution might occur post-invasion in the naïve populations of our species. In the early period, we expected the largest differences among the evolution treatments, because additional ongoing evolution after the second round of invasions would be unlikely to yet occur in naïve populations. In the late observation period, we reasoned that ongoing evolution might make the naïve populations of species more similar to evolved populations, making any differences among treatments less distinct. We used comparisons of observations from both time periods to provide us with information about the persistence of biomass patterns among treatments through the course of the experiment.

Biomass calculations

Values from Foissner and Berger (1996) provided estimates of biomass (mg) for *E. patella, P. caudatum, P. bursaria, P. niveus, S. teres,* and *S. coeruleus*. We converted values reported for weight in milligrams per 10^6 cells to weight in micrograms per cell by multiplying these tabulated values by 10^{-3}. The sum of the weights per cell of each species present multiplied by the mean number of cells per mL estimated the mean total biomass per mL for each replicate. For *E. daidaleos,* we estimated weight using the regression of weight on length for five *Euplotes* species reported in Foissner and Berger (1996). The weight of *B. americanum* was estimated proportionally from the length-weight values of a related species, *Blepharisma lateritium*. We estimated weights for *Lecane* sp. and *Monostyla* sp. from the *Euplotes* regression using measurements provided in Edmundson (1959) because they are similar in morphology (*i.e.,* disk-like). Formulas are reported in Appendix 1. Our biomass estimates assume that there were no evolutionary changes in organism size, and that changes in biomass simply reflect differences in abundance multiplied by species specific values of average biomass per individual.

Statistical analyses

A Multivariate Analysis of Variance [MANOVA SAS 9.4 (SAS Institute 2011)] for each observation period (early and late) evaluated the effect of evolution treatments on the mean of the logarithm of each resident/native species' biomass for each assemblage. In assemblage A *P. niveus* failed to establish in the –I/+R treatment, likely due to exceptionally small initial propagule size. For this resident and each invader, we instead conducted separate ANOVAs for dynamics. We assessed differences in mean total consumer biomass aggregated over all species among invasion history treatments using separate ANOVAs for each assemblage. Tukey's HSD multiple comparison test (at $\alpha = 0.05$) identified treatments that differed in pairwise comparisons.

Results

Observed biomass of invaders reflected the differences in abundances among treatments reported in Faillace and Morin (2016). Evolution by the invader resulted in increased biomass of *E. daidaleos* (early: $F_{3,16} = 97.25$, $p < 0.0001$), an effect that remained persistent throughout the course of the experiment (late: $F_{3,16} = 15.84$, $p < 0.0001$). In contrast, evolution by residents decreased the biomass of the invader *P. bursaria* in both time periods (early: $F_{3,16} = 54.80$, $p < 0.0001$; late $F_{3,16} = 13.57$, $p = 0.0001$). Biomass production of residents in both assemblages varied idiosyncratically among species, but again followed initial patterns reported previously for average abundance in Faillace and Morin (2016) (assemblage A early: $F_{20,54.06} = 16.43$, $p > 0.0001$, late $F_{20,54.06} = 9.06$, $p < 0.0001$; assemblage B early: $F_{16,52.57} = 3.80$, $p = 0.0001$, late: $F_{16,52.57} = 2.37$, $p = 0.0098$) (Figs 2 and 3). Patterns in the late observation period for both assemblages remained mostly similar, although significant differences among treatments in the early phase did not always persist in the late phase (Figs 4 and 5). For one rotifer species, *Lecane* sp. in assemblage A, significant differences among treatments were not apparent until the late observation period, at which point evolution by *Lecane* sp. appeared to increase its biomass relative to the uninvaded control (Fig. 4e).

Evolution reduced total consumer biomass in assemblage A relative to the evolutionarily naïve state (-I/-R) and the uninvaded control (-R) (early: $F_{4, 20} = 16.07$, $p < 0.0001$; late: $F_{4,20} = 6.97$, $p = 0.0011$). Invasion by naive populations of *E. daidaleos* into communities of naïve residents (-I/-R) resulted in a slight, but statistically indistinguishable decrease in mean biomass relative to uninvaded controls (-R). Mean consumer biomass was initially more than an order in magnitude higher in the uninvaded control (-R) than in the coevolved treatment (+I/+R) (Fig. 6a). The direction of changes in mean biomass were persistent over the course of our experiment; evolutionarily experienced treatments (all +I or +R treatments) produced significantly less biomass than the uninvaded controls (-R) and naïve invasions (-I/-R) retained biomass values most similar to the uninvaded controls (Fig. 6c).

Mean consumer biomass exhibited a different pattern in assemblage B. Here, biomass initially differed among the invaded treatments as a result of evolution treatments (initial: $F_{4, 20} = 6.67$, $p = 0.0014$). The naïve invasion (-I/-R) case displayed decreased biomass relative to the uninvaded controls (-R), but evolution of residents (+I/+R and -I/+R treatments) restored mean biomass to a level statistically indistinguishable from the production in the uninvaded controls (Fig. 6b). Although we detected differences between the uninvaded and invaded states in the later dynamics (later: $F_{4, 20} = 5.99$, $p = 0.0024$), the importance of initial differences in evolutionary history apparently decreased over the course of the experiment. In the later phase average consumer biomass was indistinguishable among all invaded treatments, but invaded treatments produced significantly less consumer biomass than did the uninvaded controls (Fig. 6d).

Discussion

Eco-evolutionary dynamics are emerging as an important area of study in invasion ecology. Earlier research has demonstrated that both invading and native species can exhibit apparently evolved changes in traits during the course of biological invasions (*e.g.,* Lee 2002; Strauss *et al.* 2006a; Carroll 2007a; Carroll 2007b; Mealor & Hild 2007; Phillips *et al.* 2010; Goergen *et al.* 2011; Shine 2012; Strauss 2012; Colautti & Lau 2015), but it has remained unclear what the consequences of such evolution have at the community or ecosystem level. In natural systems, long-term observations suggest that interactions among native and invading species can change as a result of evolution (Carlsson *et al.* 2010; Carthey & Banks 2015). Recent experimental evidence suggests that ongoing evolution during biological invasions has the ability to alter the consequences of invasions by changing interspecific interactions and community composition (Faillace & Morin 2016), and that evolutionary history can result in altered ecosystem properties (Palkovacs et al. 2009). Here we have demonstrated that evolution following biological invasions also affects population and community biomass, one measure of ecosystem functioning.

Results from our previous work suggested that both invading and resident species could sometimes demonstrate improved performance, in the form of increased average abundance, which we attributed to a result of evolution. In one case, ongoing evolution exacerbated the negative effects of invasion on residents, while in the other assemblage compensatory evolution by resident species resulted in increased biotic resistance to invaders. For two resident species in assemblage A, *E. patella* and *P. niveus*, we observed that evolution resulted in such reduced abundance that the species became functionally extinct within our communities.

The effects of evolution that we observed on species performance resulted in altered ecosystem functioning as assessed by biomass. In assemblage A, biomass decreased initially by more than an order of magnitude, a result primarily driven by evolution of increased performance of the invader and reduced performance by the residents. These results suggest that losses in biomass production by the residents in assemblage A were not completely offset by gains in the invader, despite the increased performance and biomass production of the invader as a result of ongoing evolution. This effect persisted through time and remained of large magnitude. One resident species in assemblage A, *Lecane* sp., initially appeared to be unaffected by evolutionary history with the invader, but showed increased biomass in the late observation period in the –I/+R treatment relative to the uninvaded control (-R) and the naïve invasion treatment (-I/-R). We scaled the course of our experimental timeline to the potentially faster evolutionary dynamics of the protist species present in our assemblages. It is possible that the slower evolutionary dynamics of rotifers relative to protists resulted in this unexpected result, however, we have not attempted to test this possibility. It is noteworthy that the naïve invasion case (-I/-R), analogous to recent invasions in nature, did not decrease biomass appreciably in comparison to the uninvaded control treatment, suggesting that long-term effects on ecosystem functioning may not be apparent when the consequences of invasions are assessed early during the invasion process.

In assemblage B, invasion also ultimately resulted in greatly reduced biomass relative to uninvaded controls. Results presented in Faillace and Morin (2016) indicated that evolution in assemblage B resulted in a community structure that favoured resident species and had reduced abundance of the invader, *P. bursaria*. Abundance and biomass data for individual species in assemblage B suggested that increased success of resident species as a result of evolution with the invader relative to the uninvaded control might result in increased total consumer biomass production. The initial comparisons, however, indicated that evolution resulted in a net restoration of ecosystem functioning following biological invasions but not increased biomass production relative to the uninvaded state. Despite the initial appearance of restored ecosystem functioning resulting from evolution, we observed in the late dynamics that only in the uninvaded treatment did biomass production remain relatively unchanged. All invaded treatments instead resulted in significantly diminished consumer biomass production irrespective of evolutionary history. This pattern appeared to be primarily driven by changes in success and biomass of the predator *S. coeruleus*, with total community biomass mirroring the results observed for *S. coeruleus*. *S. coeruleus* likely greatly influenced total biomass production compared to the other species present in assemblage B due to its relatively large size; although *S. coeruleus* initially increased in abundance as a result of ongoing evolution relative to naïve invasions, over time these gains diminished such that its biomass production decreased in all invaded treatments. Despite the appearance of increased biotic resistance in this assemblage, which intuitively might suggest a restoration of ecosystem functioning as resident species' abundances rebound, ecosystem functioning remained diminished as a result of invasion.

Eradication is infeasible for the vast majority of invading species (Lockwood et al. 2013). Evolution of traits resulting from novel selective pressures during biological invasions can significantly alter species interactions, community properties, and ecosystem functioning, making it insufficient to solely measure the traits that may be under selection during the invasion process. The growing interest in eco-evolutionary dynamics of invasions will continue provide important insight into how ongoing evolution will alter the consequences to community dynamics and ultimately, ecosystem functioning, in invaded systems during the course of biological invasions.

Figure 4.1a-d. Response in biomass production as a result of evolutionary history by both invaders.

a, early, and **c,** late observed responses for *E. daidaleos* (n = 5) and **b**, early, and **d,** late responses for *P. bursaria* (n = 5). Treatment groups are labelled on the x-axis with a “+” or “-” for experienced or naïve respectively, while an “I” or “R” refers to the invader or species in the resident community, respectively, while “S” refers to species from the source community. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.

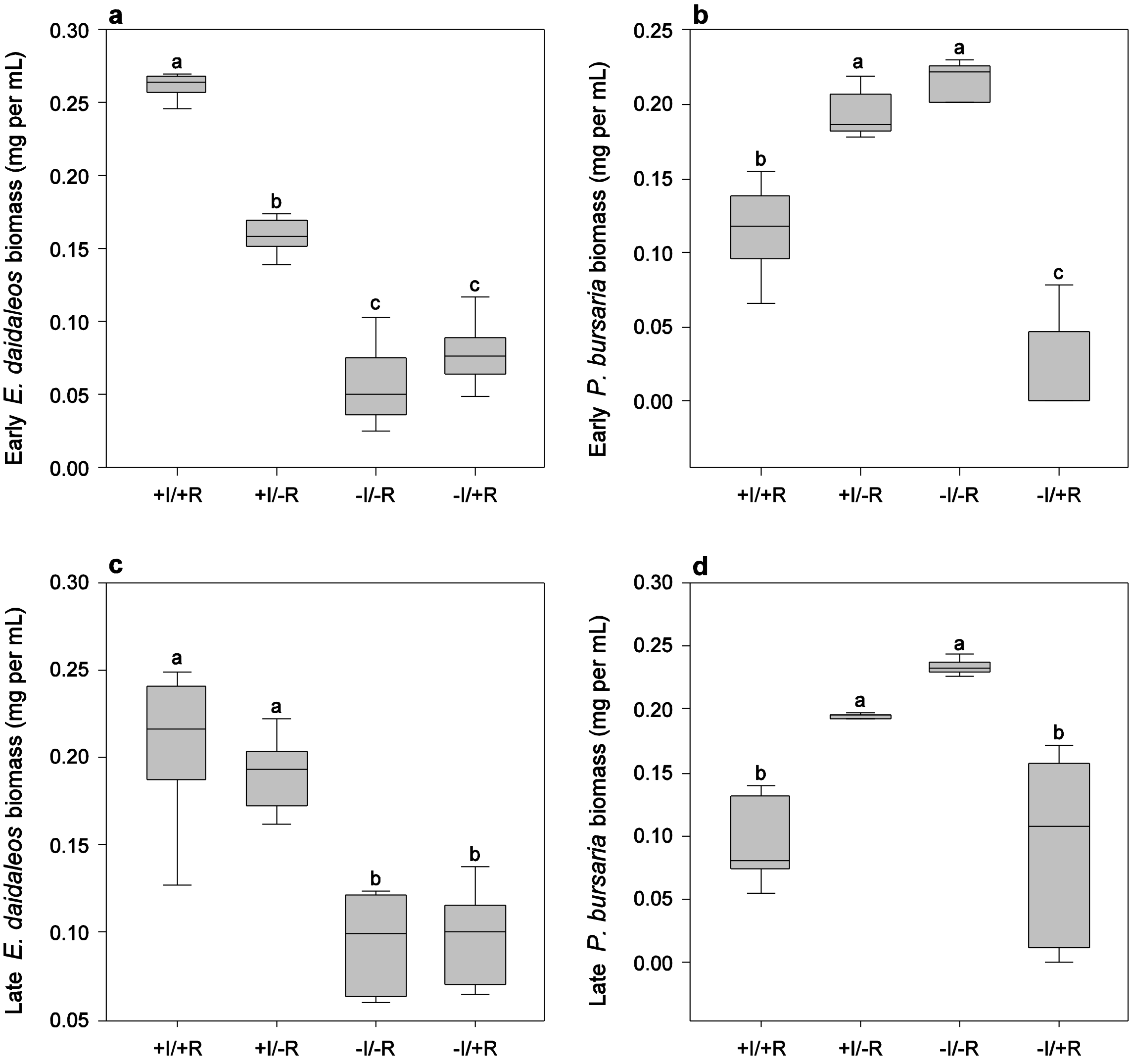

a
Early E. daidaleos biomass (mg per mL)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
a
b
c
c
+I/+R
+I/-R
-I/-R
-I/+R
b
Early P. bursaria biomass (mg per mL)
0.25
0.20
0.15
0.10
0.05
0.00
b
a
a
c
+I/+R
+I/-R
-I/-R
-I/+R
c
Late E. daidaleos biomass (mg per mL)
0.30
0.25
0.20
0.15
0.10
0.05
a
a
b
b
+I/+R
+I/-R
-I/-R
-I/+R
d
Late P. bursaria biomass (mg per mL)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
b
a
a
b
+I/+R
+I/-R
-I/-R
-I/+R

Figure 4.2a-f. Early response in biomass for resident species of assemblage A to evolutionary history with the invader, *E. daidaleos* (n = 5).

Treatment groups are labelled on the x-axis with a “+” or “-” for evolved or naïve respectively, while an “I” or “R” refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.

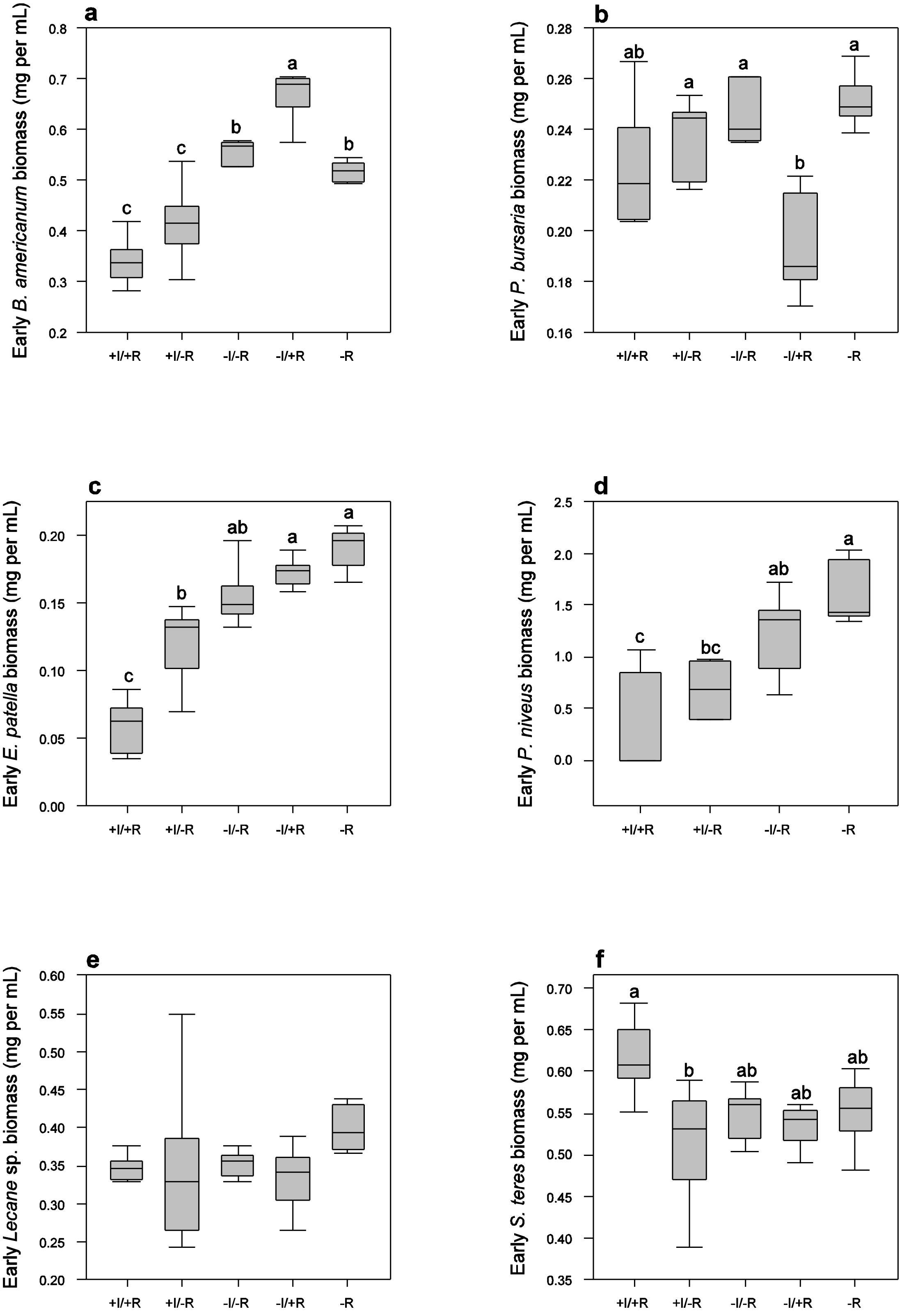

a
Early *B. americanum* biomass (mg per mL)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
c
c
b
a
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
Early *P. bursaria* biomass (mg per mL)
0.28
0.26
0.24
0.22
0.20
0.18
0.16
ab
a
a
b
a
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
Early *E. patella* biomass (mg per mL)
0.20
0.15
0.10
0.05
0.00
c
b
ab
a
a
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Early *P. niveus* biomass (mg per mL)
2.5
2.0
1.5
1.0
0.5
0.0
c
bc
ab
a
+I/+R
+I/-R
-I/-R
-R
e
Early *Lecane* sp. biomass (mg per mL)
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
+I/+R
+I/-R
-I/-R
-I/+R
-R
f
Early *S. teres* biomass (mg per mL)
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
a
b
ab
ab
ab
+I/+R
+I/-R
-I/-R
-I/+R
-R

Figure 4.3a-d. Early response in biomass for resident species of assemblage B to evolutionary history with the invader, *P. bursaria* (n = 5).

Treatment groups are labelled on the x-axis with a “+” or “-” for evolved or naïve respectively, while an “I” or “R” refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.

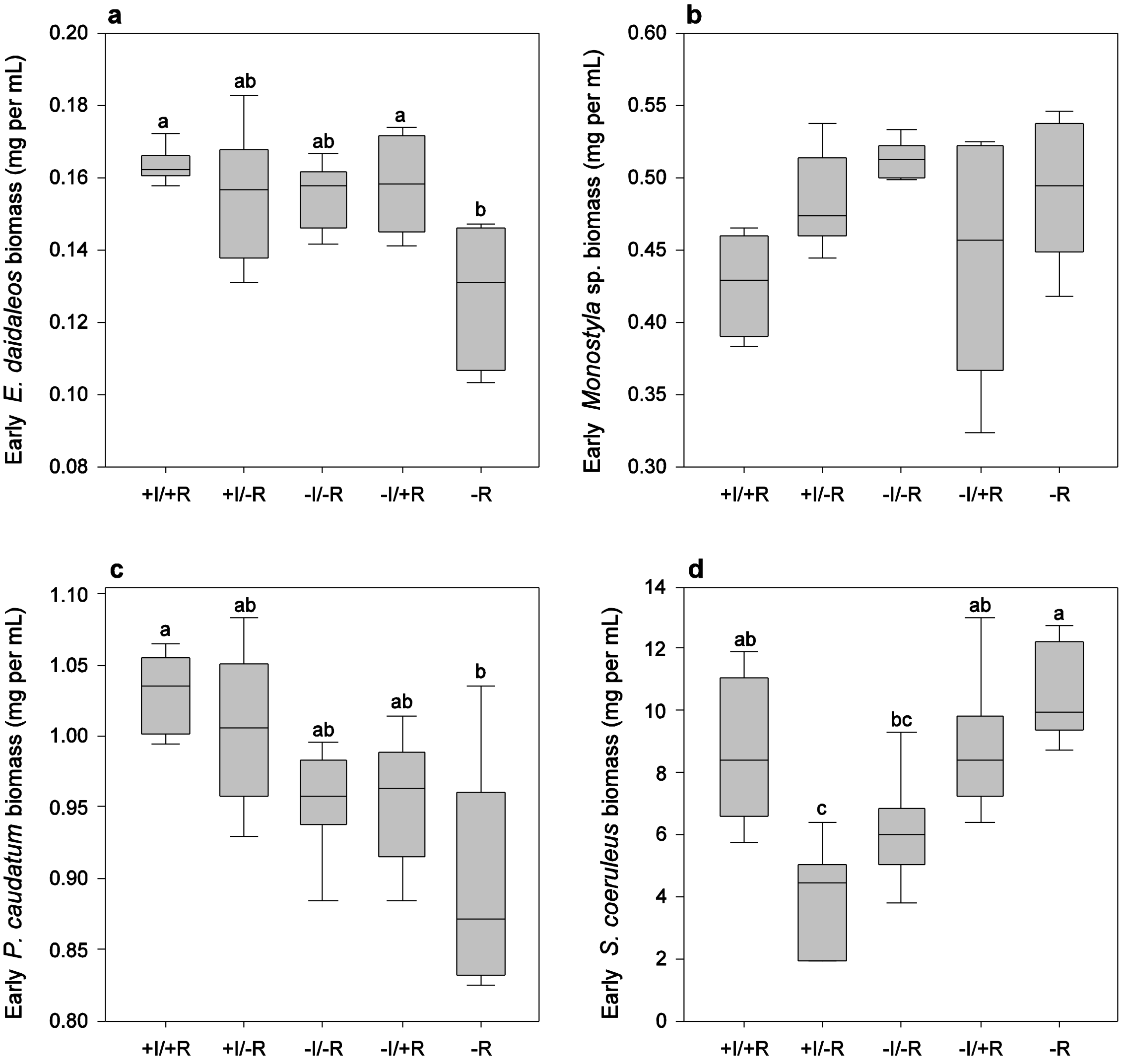

a
Early E. daidaleos biomass (mg per mL)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
a
ab
ab
a
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
Early Monostyla sp. biomass (mg per mL)
0.60
0.55
0.50
0.45
0.40
0.35
0.30
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
Early P. caudatum biomass (mg per mL)
1.10
1.05
1.00
0.95
0.90
0.85
0.80
a
ab
ab
ab
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Early S. coeruleus biomass (mg per mL)
14
12
10
8
6
4
2
0
ab
c
bc
ab
a
+I/+R
+I/-R
-I/-R
-I/+R
-R

Figure 4.4a-f. Late response in biomass for resident species of assemblage A to evolutionary history with the invader, *E. daidaleos* (n = 5).

Treatment groups are labelled on the x-axis with a “+” or “-” for evolved or naïve respectively, while an “I” or “R” refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.

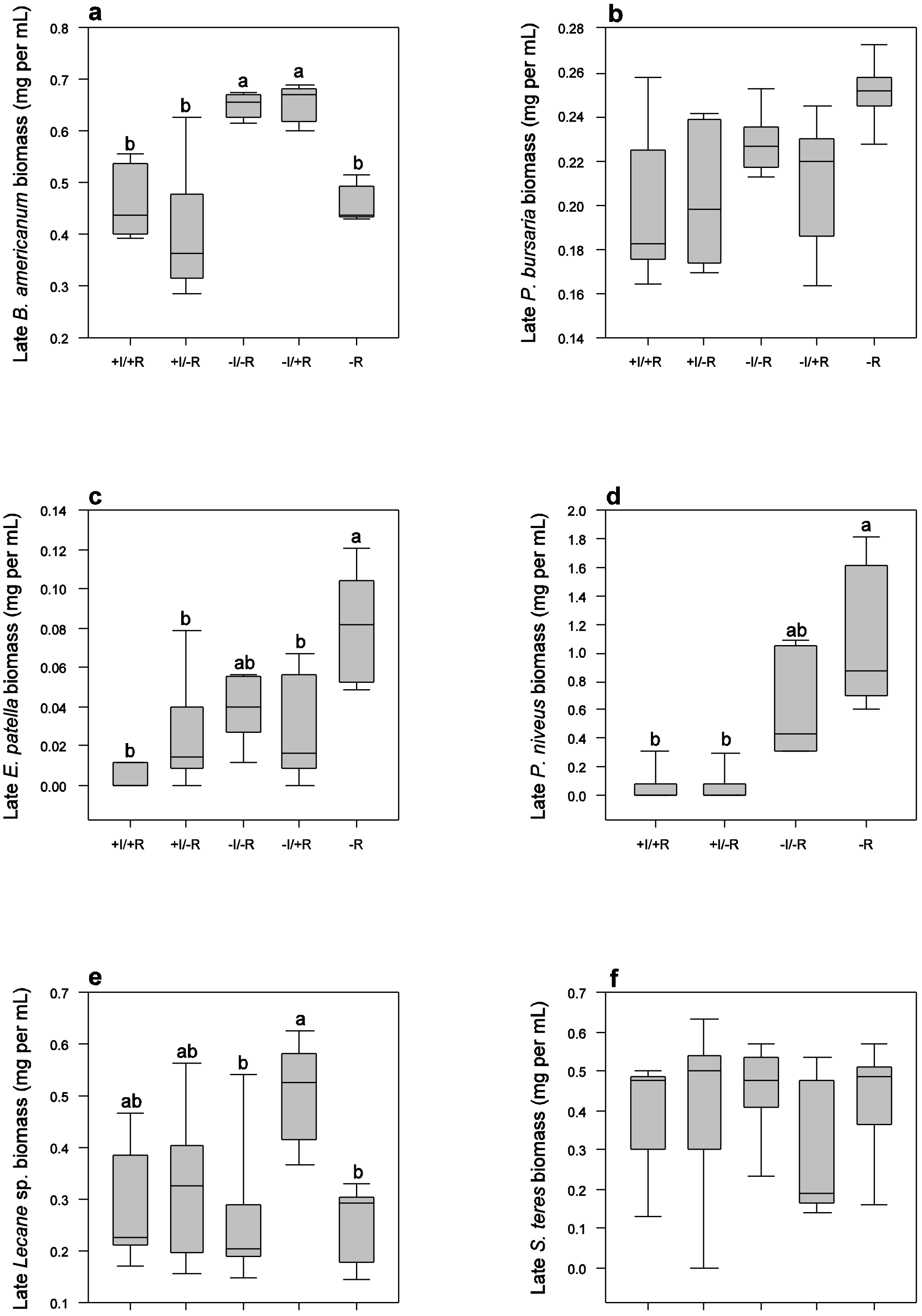

a
Late *B. americanum* biomass (mg per mL)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
b
b
a
a
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
Late *P. bursaria* biomass (mg per mL)
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
Late *E. patella* biomass (mg per mL)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
b
b
ab
b
a
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Late *P. niveus* biomass (mg per mL)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
b
b
ab
a
+I/+R
+I/-R
-I/-R
-R
e
Late *Lecane* sp. biomass (mg per mL)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
ab
ab
b
a
b
+I/+R
+I/-R
-I/-R
-I/+R
-R
f
Late *S. teres* biomass (mg per mL)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
+I/+R
+I/-R
-I/-R
-I/+R
-R

Figure 4.5a-d. Late response in biomass for resident species of assemblage B to evolutionary history with the invader, *P. bursaria* (n = 5).

Treatment groups are labelled on the x-axis with a “+” or “-” for evolved or naïve respectively, while an “I” or “R” refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.

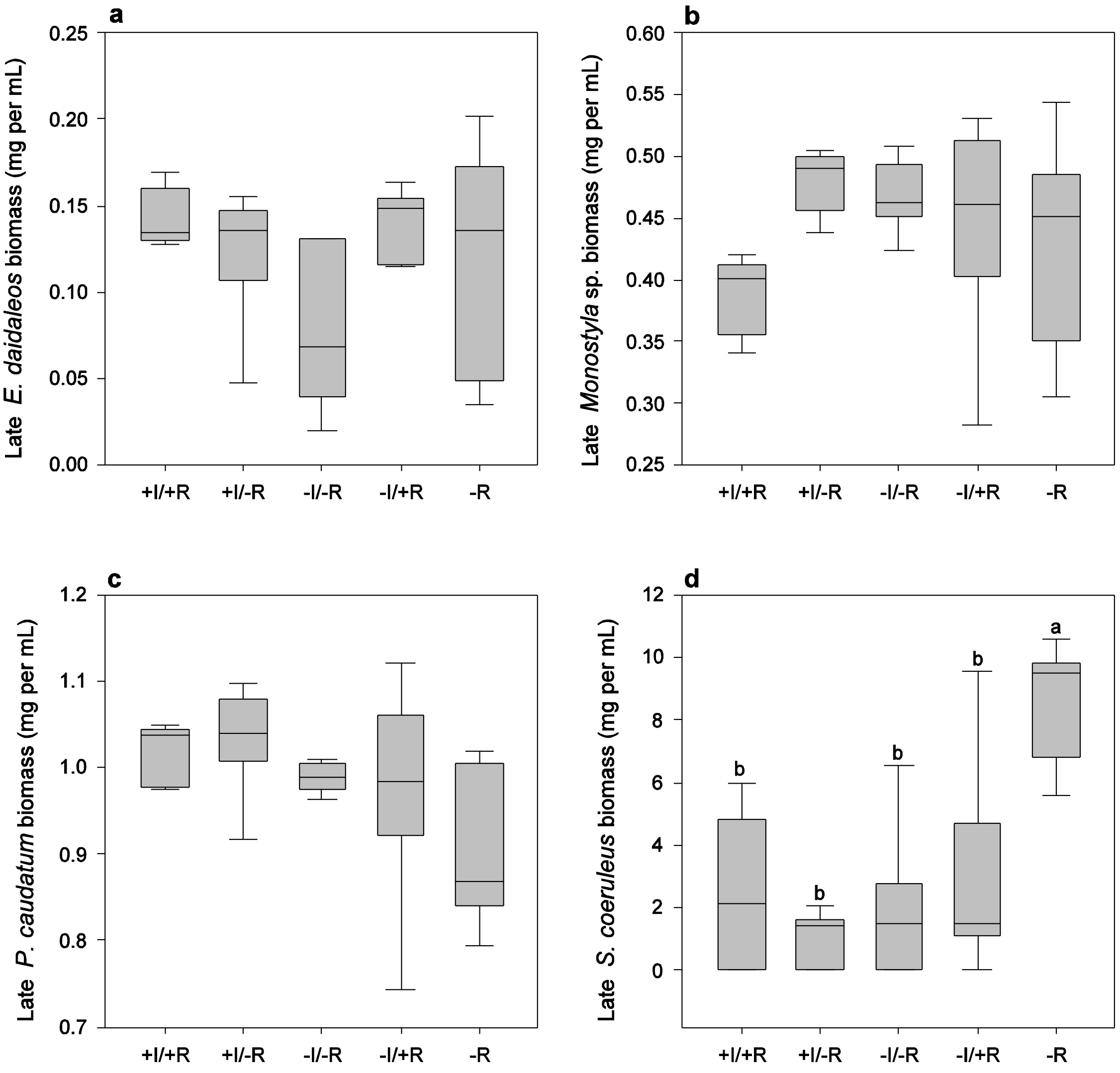
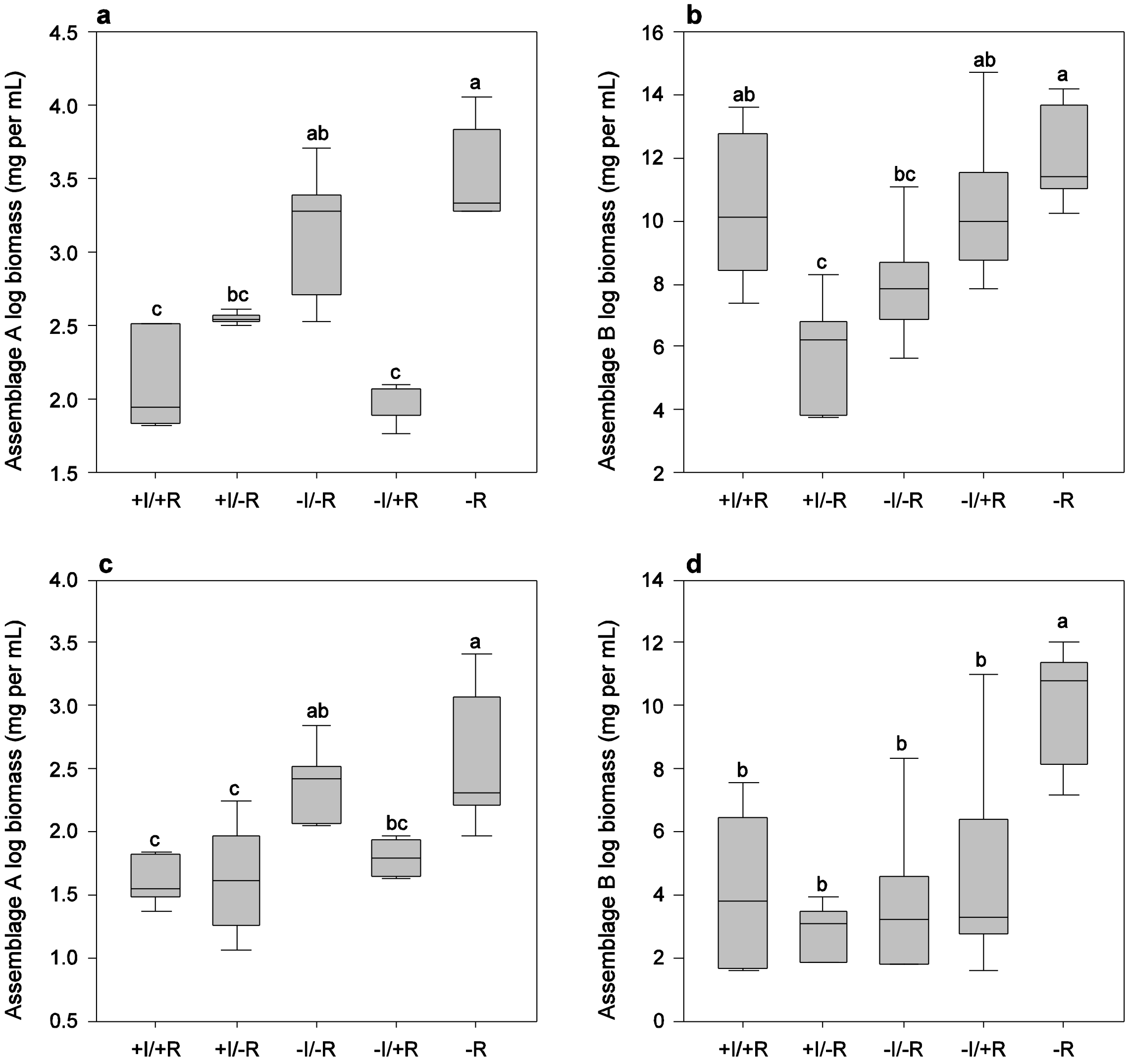

a
Late E. daidaleos biomass (mg per mL)
0.00
0.05
0.10
0.15
0.20
0.25
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
Late Monostyla sp. biomass (mg per mL)
0.25
0.30
0.35
0.40
0.45
0.50
0.55
0.60
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
Late P. caudatum biomass (mg per mL)
0.7
0.8
0.9
1.0
1.1
1.2
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Late S. coeruleus biomass (mg per mL)
0
2
4
6
8
10
12
b
b
b
b
a
+I/+R
+I/-R
-I/-R
-I/+R
-R

Figure 4.6a-d. Change in total consumer biomass as a result evolutionary history with an invader.

a, early, and **c,** late responses for assemblage A to invasion by *E. daidaleos* (n = 5). **b**, early and **d,** late response for assemblage B to invasion by *P. bursaria* (n = 5). Treatment groups are labelled on the x-axis with a “+” or “-” for evolved or naïve respectively, while an “I” or “R” refers to the invader or resident species. Treatments that differ significantly in pairwise post-hoc tests are indicated with the different small letters above the boxes. Box plots: middle line, median; box, interquartile range; whiskers, 10th and 90th percentiles.


a
Assemblage A log biomass (mg per mL)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
c
bc
ab
c
a
+I/+R
+I/-R
-I/-R
-I/+R
-R
b
Assemblage B log biomass (mg per mL)
16
14
12
10
8
6
4
2
ab
c
bc
ab
a
+I/+R
+I/-R
-I/-R
-I/+R
-R
c
Assemblage A log biomass (mg per mL)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
c
c
ab
bc
a
+I/+R
+I/-R
-I/-R
-I/+R
-R
d
Assemblage B log biomass (mg per mL)
14
12
10
8
6
4
2
0
b
b
b
b
a
+I/+R
+I/-R
-I/-R
-I/+R
-R

CONCLUSION

The causes and consequences of biological invasions vary among species and communities, making broad generalizations difficult, yet because biological invasions remain one of the most important threats to biodiversity worldwide, additional insight into both topics is sorely needed. The purpose of this dissertation was to explore invasion impacts in the context of biotic interactions with an eye to ecological and evolutionary mechanisms. By empirically testing the effects of evolutionary experience of invading and resident species in laboratory communities with short generation times, I have overcome some of the limitations of studies of biological invasions in natural communities and improved our current knowledge of the role of evolution during ongoing invasions.

In the first chapter of my dissertation, I found that exotic shrubs did not outperform native shrubs for a contested patch of soil nitrogen, but instead the native *Rubus allegheniensis* and exotic *Rubus phoenicolasius* both grew to significantly larger size than competitor plants and reduced competitor growth by greater than 50 percent. Although root competition appears to be important for these shrub species, exotic plants to not routinely outcompete native shrubs suggesting that root competition among shrubs may not be an important trait governing invasion success.

To fully understand the consequences of biological invasions, we must also understand how biological invasions affect communities over evolutionary time. The remaining chapters of my dissertation used laboratory microcosms to study how evolution among resident and invading species altered the consequences of biological invasions in experimental communities. In Chapter 2, I determined that evolution resulted in increased performance for one invader, *Euplotes daidaleos*, in an assemblage of bacteria, protists, and rotifers, while in a second assemblage, evolution resulted in the appearance of increased biotic resistance of residents against a different invader, *Paramecium bursaria*. Changes in performance of both resident and invading species also resulted in significant differences in community composition in both assemblages. In one assemblage, communities with naïve invaders were most similar, while in the other assemblage, communities with naïve residents were most similar.

In the third chapter of my dissertation, I examined how evolution altered the temporal variability in species abundances. An important assumption of theory and work tying temporal variability to stability, extinction risk, and regime shifts is that observed temporal variability results from unvarying traits of species interacting with other species or the environment rather than ongoing evolution that alters those traits. I showed that evolutionary history alone can affect temporal variation in abundance, generating important consequences for interspecific interactions among species and complicating inferences about the consequences of temporal variability in biological communities. The pattern of temporal variability in abundance for *E. daidaleos* most closely matched predictions of evolution decreasing the attack rate of predators on prey, implying that this invader may have evolved increased defense against predators that were resident species of the assemblage into which it invaded. Several resident species in this community experienced decreased abundances concurrent with increased temporal variability in abundance suggesting a means by which evolution may predispose those populations to extinction, while decreased temporal variability in invader abundance could facilitate invader persistence. These effects on community dynamics may provide one mechanism to explain how evolution can exacerbate invasions in some communities and ameliorate invasions in others.

In the final chapter of my dissertation I examined how evolutionary experience of invading and resident species affected ecosystem functioning. I examined biomass production of individual species and the entire community and determined the ongoing evolution of invading and resident species ultimately decreased biomass production, and consequently, reduced this aspect of ecosystem functioning. Patterns of biomass production mirrored the patterns of abundance of species observed in Chapter 2, but the ultimate failure of ongoing evolution to restore biomass production to that of the uninvaded state should serve as a caution for biologists and managers eager to allow “nature to take its course” in the hope that biological communities will recover from detrimental invasions in the future. While research on the eco-evolutionary dynamics of biological invasions is in its earliest stages, my dissertation provides compelling evidence that evolution can alter the consequences of biological invasions such that initial post-invasion dynamics may not adequately predict the long-term effects of invasions.

APPENDIX 1 R statistical language code used in Lotka-Volterra competition and predator-prey models (Chapter 3)

```
# Two LV competitors in a pulsed-dilution environment; corresponds to subculturing
every so many days.

# Continuous time 2 species Lotka -Volterra competition.
# This is set up to use alphas and Ks.


# The graph shows the times series for abundance of both
# competitors over time.


# The next line pauses output between graphs, when there are multiple graphs produced.
par(ask=TRUE)


lvcomp2 <- function(t, n, parms) {
        with(as.list(parms), {
                dn1dt <- r1*n[1]*(1-a11*n[1]/k1 -a12*n[2]/k1)
                dn2dt <- r2*n[2]*(1-a22*n[2]/k2 -a21*n[1]/k2)
                list(c(dn1dt,dn2dt))
        })
}

library(deSolve)

# You can change the values of model parameters below.
# r1 and r2 are the rates of increase for species 1 and 2
# a21 and a12 are the competition coefficients
# k1 and k2 are the carrying capacities for species 1 and 2

############################################################################
##

#Runs indicates the number of subculturing events that provide a regular forcing
#function for the dynamics.

runs<-20
```

```
#outall is the matrix that holds the results of the simulation.

outall<-matrix(0,nrow=(20*runs),ncol=3)
outall[1,]<-c(1,1,1)
outall

#initial gives the starting values of population size for species 1 and 2
# the program runs by setting up an initial simulation and then uses results
# from the last day to get starting conditions for subsequent runs.

initial<-c(1,1)

parms <- c(r1=0.2, r2=0.2, a11=1.0, a21=0.5, a22=1.0, a12=0.9, k1=1000, k2=1000)

out1 <- ode(y = initial, times = 1:20, func = lvcomp2, parms=parms)
matplot(out1[,1], out1[,-1], type="l", col=c(2,1), xlab="Time",ylab ="N")

outall[1:20,]<-out1
outall

#initialN holds results from the end of each simulation which are then used to
#estimate starting conditions for the next round.

initialN <- matrix(0,nrow=runs,ncol=3)
initialN[1,]<-outall[20,3]
initialN

#this loop runs the simulation for the many runs, each run starts with
#population sizes divided by 10, a ten fold dilution.

for (i in 1:(runs-1)){

        out<-ode(y = c((initialN[i,2]/10),(initialN[i,3]/10)), times =
(1+(20*(i))):(20+(20*(i))), func = lvcomp2, parms=parms)
        out
        index1<-(i)
        index1
        index2<-(1+(20*(i)))
        index2
        outall[(1+(20*(i))):(20+(20*(i))),]<-out
        outall
        initialN[(i+1),]<-out[20,]
}
```

```
initialN
outall

# This graphs the dynamics.

matplot(outall[,1],log10((outall[,-1])), type="l", lty=1:2, col=c(2,1), xlab="Time",ylab
="N")
legend("bottomright", c("Species 1","Species 2"), lty=2:1, col=c(1,2), bty="n")
dev.copy2eps(file="lvcomp9.eps", width=4)

#estimates of the temporal variation in abundance over time

# species 1
mean1<-mean(log10(outall[,2]))
mean1
sd1<-sd(log10(outall[,2]))
sd1
cv1<-(sd(outall[,2])/mean(outall[,2]))*100
cv1

#species 2
mean2<-mean(log10(outall[,3]))
mean2
sd2<-sd(log10(outall[,3]))
sd2
cv2<-(sd(outall[,3])/mean(outall[,3]))*100
cv2



########################################################################
########

# create a function for the Lotka-Volterra predator-prey model, with logistic
# regulation of the prey

par(ask=TRUE)

library(deSolve)

# set parameter values for the model, b - prey rate of increase, a - attack rate
# e - efficiency of conversion of prey into predators, s - predator death rate,
# k - prey carrying capacity
```

```
b<- 1.0
a<- 0.3
e<- 0.1
s<- 0.5
k<- 100

# define the function used in ode

predpreyLVK <- function (t, y, params){
        H <- y[1]
        P <- y[2]
        with(as.list(params), {
                dH.dt <- b*H*(1-H/k) - a*P*H
                dP.dt <- e*a*P*H - s*P
                return(list(c(dH.dt, dP.dt)))
        })
}


##########################################################################
#Runs indicates the number of subculturing events that provide a regular forcing
#function for the dynamics.

runs<-20

#outall is the matrix that holds the results of the simulation.

outall<-matrix(0,nrow=(20*runs),ncol=3)
outall[1,]<-c(1,25,5.62)

params1 <- c(b=b, a=a, s=s, e=e, k=k)
Time <- seq(1,20, by = 1)
LVK.out <- ode(c(H0 = 25, P0 = 5.62), Time, predpreyLVK, params1)
matplot(Time, log10(LVK.out[,2:3]), type="l", ylab = "Population Size", ylim=c(0,2))
legend("topright", c("Prey-H","Predator-P"), lty=1:2, bty="n")


#initial gives the starting values of population size for species 1 and 2
# the program runs by setting up an initial simulation and then uses results
# from the last day to get starting conditions for subsequent runs.

initial<-c(25,5.62)
```

```
out1 <- LVK.out
out1
outall[1:20,]<-out1
outall

#initialN holds results from the end of each simulation which are then used to
#estimate starting conditions for the next round.

initialN <- matrix(0,nrow=runs,ncol=3)
initialN[1,]<-outall[20,]
initialN

#this loop runs the simulation for the many runs, each run starts with
#population sizes divided by 10, a ten fold dilution.

for (i in 1:(runs-1)){

	out<-ode(y = c((initialN[i,2]/10),(initialN[i,3]/10)), times =
(1+(20*(i))):(20+(20*(i))), predpreyLVK, params1)
	out
	index1<-(i)
	index1
	index2<-(1+(20*(i)))
	index2
	outall[(1+(20*(i))):(20+(20*(i))),]<-out
	outall
	initialN[(i+1),]<-out[20,]
}

initialN
outall

# This graphs the dynamics.

matplot(outall[,1],log10(outall[,-1]), type="l", col=c(2,1), xlab="Time",ylab ="N")
legend("bottomright", c("Prey-H","Predator-P"), lty=1:2, bty="n")
dev.copy2eps(file="lvpredprey3.eps", width=4)


#estimates of the temporal variation in abundance over time
#prey stats
meanH<-mean(log10(outall[,2]))
meanH
sdH<-sd(log10(outall[,2]))
```

```
sdH
cvH<-(sd(outall[,2])/mean(outall[,2]))*100
cvH
#predator stats
meanP<-mean(log10(outall[,3]))
meanP
sdP<-sd(log10(outall[,3]))
sdP
cvP<-(sd(outall[,3])/mean(outall[,3]))*100
cvP
```

APPENDIX 2 Supplementary Table 3.3

Supplemental Table 3.3. Partial Pearson's correlation coefficients between species' mean abundance and standard deviation of mean abundance for all residents and invaders in assemblages A and B for both early and late dynamics.

Assemblage	Species	Sample size (n)	Early Correlation	p value	Late Correlation	p value
A	*Blepharisma americanum*	25	-0.39292	0.0520	-0.03810	0.8565
A	*Euplotes daidaleos* (invader)	25	-0.67728	0.0010	-0.65390	0.0018
A	*Euplotes patella*	25	-0.47428	0.0166	0.60272	0.0014
A	*Lecane* sp.	25	-0.10288	0.6246	-0.18528	0.3753
A	*Paramecium bursaria*	25	-0.27063	0.1907	-0.55282	0.0042
A	*Prorodon niveus*	25	0.63948	0.0006	0.91341	<0.0001
A	*Spirostomum teres*	25	-0.52590	0.0069	0.00813	0.9692
B	*Euplotes daidaleos*	25	-0.42386	0.0347	-0.31659	0.1231
B	*Monostyla* sp.	25	-0.24894	0.2301	-0.45990	0.0207
B	*Paramecium bursaria* (invader)	25	-0.02059	0.9313	-0.00949	0.9683
B	*Paramecium caudatum*	25	-0.15131	0.4703	-0.81180	<0.0001
B	*Stentor coeruleus*	25	-0.06675	0.7512	0.86858	<0.0001

APPENDIX 3 Biomass calculations for species in assemblages A and B and total consumer biomass calculation for each assemblage (Chapter 4)

Biomass for *Blepharisma americanum* = *B. americanum* mean abundance x 0.317
Biomass for *Euplotes daidaleos* = *E. daidaleos* mean abundance x 0.112
Biomass for *Euplotes patella* = *E. patella* mean abundance x 0.093
Biomass for *Lecane* sp. = *Lecane* sp. mean abundance x 0.219
Biomass for *Monostyla* sp. = *Monostyla* sp. mean abundance x 0.219
Biomass for *Paramecium bursaria* = *P. bursaria* mean abundance x 0.12
Biomass for *Paramecium caudatum* = *P. caudatum* mean abundance x 0.5
Biomass for *Prorodon niveus* = *P. niveus* mean abundance x 2.5
Biomass for *Spirostomum teres* = *S. teres* mean abundance x 0.380
Biomass for *Stentor coeruleus* = *S. coeruleus* mean abundance x 12

Total biomass for Assemblage A = (*B. americanum* mean abundance x 0.317) + (*E. daidaleos* mean abundance x 0.112) + (*E. patella* mean abundance x 0.093) + (*Lecane* sp. mean abundance x 0.219) + (*P. bursaria* mean abundance x 0.12) + (*P. niveus* mean abundance x 2.5) + (*S. teres* mean abundance x 0.380)

Total biomass for Assemblage B = (*E. daidaleos* mean abundance x 0.112) + (*Monostyla* sp. mean abundance x 0.219) + (*P. bursaria* mean abundance x 0.12) + (*P. caudatum* mean abundance x 0.5) + (*S. coeruleus* mean abundance x 12)

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