



An experimental test of stabilizing forces in the field niche

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Abstract. Modern coexistence theory holds that stabilizing mechanisms, whereby species limit the growth of conspecifics more than that of other species, are necessary for species to coexist. Here, we used experimental and observational approaches to assess stabilizing forces in eight locally co-occurring, annual, legume species in the genus *Trifolium*. We experimentally measured self-limitation in the field by transplanting *Trifolium* species into each other's field niches while varying competition and related these patterns to the field coexistence dynamics of natural *Trifolium* populations. We found that *Trifolium* species differed in their responses to local environmental gradients and performed best in their home environments, consistent with habitat specialization and presenting a possible barrier to coexistence at fine scales. We found significant self-limitation for 5 of 42 pairwise species combinations measured experimentally with competitors absent, indicating stabilization through plant–soil feedbacks and other indirect interactions, whereas self-limitation was largely absent when neighbors were present, indicating destabilizing effects of direct plant–plant interactions. The degree of self-limitation measured in our field experiment explained year-to-year dynamics of coexistence by *Trifolium* species in natural communities. By assessing stabilizing forces and environmental responses in the full n -dimensional field niche, this study sheds light on the roles of habitat specialization, plant–soil feedbacks, and plant interactions in determining species coexistence at local scales.

Key words: coexistence; competition; co-occurrence; niche differences; plant–soil feedback; stabilization.

INTRODUCTION

That species must differ in order to coexist is one of the most important ideas in the study of diversity (Darwin 1859, Grinnell 1904, Gause 1932), yet local communities often contain many ecologically similar species. Understanding whether and how these species stably coexist is an ongoing challenge for ecology. According to modern coexistence theory, coexistence requires that stabilizing niche differences, which cause species to limit conspecific individuals more strongly than they limit individuals of other species, exceed relative fitness differences that drive competitive exclusion (Chesson 2000a, Levine and HilleRisLambers 2009). Stabilizing niche differences boost the performance of rare species, allowing them to recover from low densities, while limiting the performance of species as they become relatively more abundant (i.e., negative frequency dependence), preventing them from excluding their competitors (Adler et al. 2007, Levine et al. 2008).

The stabilizing mechanisms that maintain coexistence operate on multiple spatial scales (Chesson 2000b, Amarasekare 2003). In spatially heterogeneous environments, species with different responses to spatially varying environmental variables can coexist through spatial niche partitioning so long as trade-offs in the ability to exploit different environments allow each species to have a competitive advantage in some sites (Kneitel and Chase 2004). Such spatial niche differences can maintain coexistence at the among-habitat scale but may hinder coexistence at fine (within-habitat) scales by increasing the relative fitness advantage of resident species over invaders in their “home” environments.

Within spatially homogeneous competitive environments, coexistence typically requires stabilizing feedbacks arising from niche differences that cause species to limit themselves more strongly than they limit other species. Classical examples of stabilizing feedback mechanisms include resource partitioning, which causes species to compete more strongly with conspecifics than with heterospecifics (Hutchinson 1959, MacArthur 1970, Tilman 1982), and specialized enemies such as predators and pathogens (Janzen 1970, Connell 1971). More recently, it has been proposed that shared mutualists may also act as a stabilizing force if the mutualists

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cultivated by a host species provide relatively greater benefit to that species' competitors than to the host itself (Bever 2002, Van Der Heijden et al. 2008, Lee and Inouye 2010).

Despite these important developments in coexistence theory, empirically testing specific stabilizing mechanisms and linking them to coexistence dynamics in natural communities remain a challenge. Measuring stabilization requires estimating the strength of intraspecific and interspecific interactions for each species pair in a community (Chesson 2008). One approach for empirically assessing stabilization is fitting population models to long-term demographic data for natural populations. For example, Adler et al. (2010), simulating the dynamics of a sagebrush steppe plant community using a population model parameterized by long-term observational data, demonstrated strong stabilizing niche differences between species that were enough to overcome weak average fitness differences and maintain coexistence. While useful, this approach requires measuring the performance of each species in neighborhoods that have a range of densities of conspecifics and of each competing species, which may not be feasible in very diverse natural communities. In addition, while this approach can quantify the overall strength of stabilization, it cannot isolate specific stabilizing mechanisms. A second approach that has been used to test for stabilizing effects in plant communities involves experimentally assembling communities in controlled greenhouse or common garden settings (Kraft et al. 2015, Matías et al. 2018). While providing many insights, such experiments do not include the full range of niche dimensions experienced by plants in natural communities, and therefore may miss potentially important stabilizing mechanisms. We aimed to complement the results of these studies with experiments testing coexistence in the full field niche.

Here, we used experimental and observational approaches to assess stabilizing forces in eight congeneric, annual legume (*Trifolium*) species. These *Trifolium* species occupy similar habitats in coastal grasslands in northern California and share mutualistic N-fixing rhizobial mutualists. Previous studies have shown that these species co-occur at regional to local scales (Parker and Gilbert 2007), with some species showing positive spatial associations at very fine scales (within 2-m² plots) over 15 yr, suggesting stable coexistence (Siefert et al. 2018). We experimentally tested stabilization in the field by transplanting *Trifolium* species into each other's field niches, defined as patches where each species was very abundant. We then related experimentally estimated stabilizing effects to coexistence dynamics of natural *Trifolium* populations using observational data in field plots over two years. Specifically, we ask: (1) Do *Trifolium* species differ in their response to the environment, indicating spatial niche partitioning? (2) Do *Trifolium* species exhibit stabilizing self-limitation through soil feedbacks or direct plant–plant

interactions? (3) Does the strength of self-limitation estimated in our field experiment predict coexistence dynamics of species pairs in natural communities?

METHODS

Study system

Our study focused on eight native, annual legume (*Trifolium*) species for which we have a 15-yr record of co-occurrence in coastal grasslands at Bodega Marine Reserve (BMR), California, USA. *Trifolium* form mutualistic associations with the nitrogen-fixing soil bacterium *Rhizobium leguminosarum* biovar *trifolii*. Rhizobial strains are shared across *Trifolium* species at BMR (Siefert et al. 2018), fulfilling a requirement for stable coexistence mediated by mutualists in mathematical models (Bever 1999, Umbanhowar and McCann 2005).

Field experiment to assess stabilization in the field niche

We tested self-limitation in the field by transplanting each *Trifolium* species into the field niches of each species and assessing the effects of conspecific and congeneric residents on performance. We planted 12 individuals per species into 16 field sites spanning a range of *Trifolium* density and relative species abundance at BMR. To assess the effects of resource competition and other direct plant–plant interactions on performance and self-limitation, we removed competitors from the neighborhoods of half the replicates. In sum, the experimental design consisted of 8 species \times 16 sites \times 2 competition treatments \times 6 replicates/treatment = 1,536 individuals. The experiment was repeated in two growing seasons (2014–2015 and 2015–2016) to replicate across differences in weather and other contingencies.

We selected 16 experimental sites in native *Trifolium* habitats at Bodega Marine Reserve. Sites were selected to include areas of high density of each study species. Because *Trifolium* species co-occur at fine scales within the study area, each site contained two to six *Trifolium* species. Two species, *T. microcephalum* and *T. willdenovii*, had low natural densities during the years of the experiment, so we were not able to assess their effects as residents. The remaining species each occurred in at least six sites. We excluded one site in 2015–2016 due to low survival (10%) in the previous year, resulting in 15 sites in 2015–2016. In December of each growing season, we transplanted 12 seedlings, germinated in a greenhouse in sterilized potting soil, of each species into each site. Transplanting was timed so that experimental seedlings were in a similar stage of development (mostly one to four leaves) as resident *Trifolium* seedlings. Seedlings were transplanted into microsites with at least three resident *Trifolium* seedlings growing within a 7.5-cm radius (“neighborhood” hereafter), with a minimum distance of 20 cm between transplants. Due to spatial variation in resident *Trifolium* density, the total area of sites varied

from 18 to 45 m². Individuals that did not survive transplanting were replaced with greenhouse-germinated seedlings within 2–4 weeks of the original planting date.

We randomly selected half the replicates of each species within each site to receive the neighbor removal treatment, which consisted of removing all plants within a 7.5-cm radius of the focal plant. We weeded neighborhoods by hand every 2–3 weeks during the experiment to maintain the absence of neighbors. In 2014–2015, due to an exceptional drought, we hand-watered plants four times over the course of the growing season to reduce mortality.

To characterize natural *Trifolium* and plant communities in each site, we recorded *Trifolium* species densities and visually estimated grass, forb, and legume cover within the neighborhood of each individual (excluding individuals from the neighbor removal treatment, since weeding occurred when plants were too small to be confidently identified) in April 2015 and 2016. We averaged these values over all neighborhoods within a site to obtain site mean *Trifolium* density and non-*Trifolium* community cover values. We calculated the frequency of each *Trifolium* species in each site as the density of that species as a percentage of total *Trifolium* density. To characterize site edaphic properties, we collected soil samples (approximately 10 × 10 × 10 cm) from three locations per site and pooled samples for analysis of pH, organic matter, total exchange capacity, exchangeable phosphorus, calcium, sodium, iron, manganese, and available nitrogen (ammonium and nitrate) concentrations, and sand, silt, and clay content (see Appendix S1 for soil analysis methods).

In late April to early May of each year, 18–20 weeks after planting, we harvested the transplants. We recorded survival and the number of inflorescences and measured aboveground biomass on all plants in 2015 and 2016.

Data analysis

Environmental niche partitioning.—To assess whether *Trifolium* species were specialized to different habitats, we performed a partial constrained correspondence analysis (CCA) with site mean aboveground biomass of the eight *Trifolium* species as the response matrix, site-level grass and forb cover and edaphic variables as predictors, and year as a conditioning variable. We ran separate analyses on plants from the neighbor removal and non-removal treatments. For each analysis, we implemented ANOVA-like permutation tests for joint effects of the environmental predictors (Legendre et al. 2011). A significant test indicates that the environment explains variation in the relative performance of *Trifolium* species across sites, indicating habitat partitioning or specialization among the *Trifolium* species. We tested the significance of individual environmental variables using similar permutation tests.

We also assessed whether *Trifolium* species performed better in sites more similar to their home habitat. If

similarity to home environment increases performance, then there may be a barrier to coexistence at the within-site scale, because resident species would have a relative fitness advantage over invaders in their home sites. To quantify each species' "home" environment, we performed a CCA of site-level density of resident *Trifolium* species (i.e., average *Trifolium* species densities within neighborhoods of transplants in each site) with site-level grass and forb cover and edaphic variables as predictors. We defined the "home" environment of each *Trifolium* species by its position in the resulting environmentally constrained ordination space. We measured the distance to home environment for each *Trifolium* species in each experimental site and year by projecting sites onto the ordination space and calculating the Euclidean distance from each species' home environment. Finally, we tested the effect of distance to home environment on the growth of *Trifolium* transplants using generalized linear mixed models (GLMMs) with a compound Poisson (Tweedie) distribution and log link function. We modeled the aboveground biomass of transplants as a function of species, neighbor removal treatment, environmental distance, and their interactions, with year as a fixed covariate and site as a random effect.

Growth models.—We assessed the effects of resident *Trifolium* communities on transplant growth by comparing four models. The first model included effects of biotic and abiotic habitat variables but omitted effects of resident *Trifolium* communities (hereafter, *Trifolium*-independent model). The *Trifolium*-independent model included effects of year, focal species, neighbor removal treatment, and habitat variables (site-level grass, forb, and legume cover and edaphic variables). We compared models containing all possible subsets of habitat variables using AIC_c to select the most parsimonious *Trifolium*-independent model. These effects were included in all subsequent models to provide a baseline for testing resident *Trifolium* effects in the other models. We note that resident *Trifolium* effects in the neighbors-present treatment could arise from a combination of direct plant–plant interactions as well as plant–soil feedbacks and other indirect effects, whereas resident *Trifolium* effects in the neighbor removal treatment should reflect plant–soil feedbacks and other historical effects only.

The second model examined the effects of total *Trifolium* resident density (hereafter *Trifolium*-density model), and included effects of site-level *Trifolium* density, focal species, neighbor-removal treatment, and their interactions, as well as the habitat variables identified in the *Trifolium*-independent model above. Because many coexistence models rely on negative frequency dependence and advantages when rare, the third model (hereafter, frequency-dependence model) included the effect of site-level conspecific frequency of the focal *Trifolium* species (density of the focal *Trifolium* species divided by total *Trifolium* density) in addition to all effects in the density model. The fourth model assessed pairwise

interactions between *Trifolium* (hereafter, species-interactions model) by including effects of site-level individual *Trifolium* species densities along with effects in the *Trifolium*-independent model.

For all models, we modeled aboveground biomass of *Trifolium* transplants using GLMMs with the predictors described above as fixed effects and site as a random effect, using a compound Poisson error distribution and log link function. For each type of model, we first fit a full model that included the three-way interaction (and nested two-way interactions) between focal species, neighbor removal, and resident *Trifolium* variables. We then tested the significance of interactions using likelihood ratio tests and built a parsimonious model including all simple effects and significant interactions (at $\alpha = 0.05$). We compared the resulting best model from the four model types using the Akaike information criterion with correction for small sample sizes (AIC_c), with ΔAIC_c values calculated by subtracting the minimum AIC_c value from the AIC_c of each model, and Akaike weights indicating the level of support for each model.

Because two of the eight focal species (*T. willdenovii* and *T. microcephalum*) had resident densities too low to estimate frequency effects, we fit the four models using only the six remaining focal species. To estimate effects of resident *Trifolium* species on *T. willdenovii* and *T. microcephalum*, we refit the pairwise species interactions model (model 4) using all eight focal species.

Self-limitation.—To assess the potential for *Trifolium* species to increase when rare, we estimated the degree of self-limitation by each resident species facing each congeneric invader in a pairwise manner (Eppinga et al. 2018). Using the species interactions model (model 4), we estimated the effects of each resident species j on the performance of conspecific transplants (α_{jj}) and transplants of each congeneric species i (α_{ij}). We then calculated an index of self-limitation for each resident–invader combination as the difference between the effect of the resident on the invading species and the effect of the resident on itself ($\alpha_{ij} - \alpha_{jj}$), with positive values indicating stabilizing self-limitation (resident limits itself more than the invader, or facilitates the invader more than itself) and negative values indicating destabilizing “invader-limitation” (resident limits the invader more than itself, or facilitates itself more than the invader). For each species pair, we examined whether each was self-limiting with respect to the other (i.e., mutual self-limitation), a requirement for stable coexistence (Broekman et al. 2019). We separately estimated self-limitation for each species pair with neighbors present or absent. Self-limitation in the absence of neighbors should primarily reflect soil-mediated feedbacks and other historical or legacy effects, whereas self-limitation in the presence of neighbors reflects feedbacks through direct competition and facilitation as well as soil-mediated effects. We obtained confidence intervals on interaction coefficients and self-limitation index values by

parametric bootstrapping with 10,000 replications. We tested whether self-limitation differed between neighbor removal treatments across all species combinations using a Wilcoxon signed-rank test. Because species combinations were not independent, we obtained P values using a permutation test that compared the observed Wilcoxon test statistic to a null distribution generated by shuffling neighbor treatments within species combinations (10,000 replications).

Coexistence and self-limitation in natural communities.—We wanted to explore how our field transplant experimental results and models related to natural patterns of *Trifolium* coexistence in the field. To estimate *Trifolium* species interactions and self-limitation in natural communities, we surveyed *Trifolium* species densities in 265 4-m² plots arranged along 19 transects in *Trifolium* habitats in BMR in May 2015 and 2016. None of the experimental sites were located in these plots. In each plot, we recorded presence of *Trifolium* species in the entire plot and counted *Trifolium* individuals in five 0.09-m² subplots located at the center and four corners of the plot. Based on these data, we estimated *Trifolium* species densities (individuals/m²) at the plot level.

To estimate intraspecific and interspecific interactions among *Trifolium* species, we used a neighborhood modeling framework (Kunstler et al. 2016) to model year-to-year changes in *Trifolium* species densities as a function of a species’ intrinsic growth rate, modified by effects of intra- and interspecific neighbors. Specifically, we assumed a relationship of the form

$$N_{i,p,16} = N_{i,p,15} \lambda_i \exp \left(\sum_{j=1}^S \alpha_{ij} N_{j,p,15} \right)$$

where $N_{i,p,16}$ is the density of species i in plot p in 2016, λ_i is the intrinsic growth rate of species i in the absence of competition, α_{ij} is the per-capita effect of species j on the growth rate of species i (negative values indicating competition and positive values indicating facilitation), and $N_{j,p,15}$ is the density of species j in plot p in 2015. For each *Trifolium* species with sufficient density in field plots ($N = 6$), we fit the linearized version of this equation (Kunstler et al. 2016) as a generalized linear mixed model with transect as a random effect, assuming normally distributed errors, using the lmer function in the lme4 package in R (Bates et al. 2015). Using the resulting interaction coefficients, we calculated a self-limitation index for each resident–invader combination as the difference between the resident species’ effect on the invader and its effect on itself. We obtained confidence intervals on species interaction coefficients and self-limitation index values by parametric bootstrapping with 10,000 replications.

To test whether results of our field experiment could explain coexistence dynamics in natural communities, we tested the correlation between self-limitation matrices estimated from the field experiment (with neighbors

present or removed) and from natural communities. Because species combinations were not independent, we obtained P values using a permutation test that compared the observed correlation to a null distribution generated by randomly permuting the rows and columns of one matrix and recalculating the correlation (analogous to a Mantel test), with 10,000 replications.

The data and code to produce the analyses are available on Zenodo (see *Data Availability*).

RESULTS

Habitat partitioning

Results of constrained ordination analysis showed that the relative performance of *Trifolium* species in our field transplant experiment was significantly affected by habitat variables, indicating that *Trifolium* species are specialized to different microniches within broadly similar habitat. We found significant overall effects of habitat variables with neighbors present ($P = 0.001$) and with neighbors removed ($P = 0.002$). When testing individual habitat variables, we found that *Trifolium* species were significantly differentiated in their responses to soil organic matter, P, and Na in both competition treatments, as well as their responses to grass cover, sand content, and soil pH with neighbors removed (Appendix S1: Fig. S1).

Trifolium transplants generally performed better in sites more similar to their home habitat, although the strength of this effect depended on species and presence of neighbors (three-way species \times environmental distance \times neighbor removal effect, $\chi^2_3 = 13.7$, $P = 0.017$; Appendix S1: Fig. S2). When averaged across focal species, distance to home environment had a significant negative effect on growth of transplants with neighbors present ($t = -2.32$, $P = 0.02$) but no effect with neighbors removed ($t = 1.13$, $P = 0.26$).

Trifolium density and frequency effects on transplant performance

Performance of transplanted *Trifolium* individuals varied significantly between the two years in which the field experiments were conducted ($\chi^2_1 = 63.5$; $P < 0.001$). Survival of *Trifolium* transplants was 37% in 2015 (dry year) and 68% in 2016. Survival varied among species, ranging from 44% for *T. willdenovii* to 64% for *T. fucatum*. Mean aboveground biomass was almost 10 times greater in 2016 (0.22 g) than in 2015 (0.028 g), reflecting greater rainfall in 2016. Across all years and species, aboveground biomass was positively correlated with site grass and forb cover, soil N, pH, and sand content, and negatively correlated with soil P. Neighbor removal increased aboveground biomass of transplants by 98% on average (95% CI = 77–127%), with the strength of the effect varying among species ($\chi^2_3 = 14.8$; $P = 0.01$).

All models of aboveground biomass that included effects of resident *Trifolium* communities outperformed

the *Trifolium*-independent model ($dAIC_c > 20$), indicating that resident *Trifolium* communities affected transplant growth through some combination of direct competitive or facilitative interactions, soil feedbacks, and other indirect effects (Appendix S1: Table S1). The model including all pairwise interactions between *Trifolium* species (model 4) received the strongest support (AIC_c weight = 0.80), followed by the frequency dependence model (model 3) (AIC_c weight = 0.20).

Overall, transplants performed better in sites with greater resident *Trifolium* density ($t = 2.2$, $P = 0.03$; Appendix S1: Fig. S3), with the strength of the effect varying among species ($\chi^2_5 = 20.4$; $P = 0.001$) but not between neighbor removal treatments ($\chi^2_1 = 0.01$; $P = 0.99$).

Transplants also generally performed better in sites with higher frequency of conspecifics, indicating positive frequency dependence, and these effects varied marginally among species and between neighbor removal treatment (three-way species \times frequency \times neighbor removal interaction, ($\chi^2_3 = 8.27$; $P = 0.14$). Averaged across species, there was significant positive frequency dependence with neighbors present ($t = 0.33$, $P = 0.001$), and three of six species (*T. bifidum*, *T. fucatum*, and *T. macraei*) had significant positive frequency dependence individually (Fig. 1). The strength of frequency dependence became weaker, and even marginally negative for some species, in the absence of neighbors ($t = 1.96$, $P = 0.05$). This result indicates that frequency-dependent feedbacks through soil and other indirect effects in the absence of neighbors were generally neutral, whereas feedbacks through direct plant–plant interactions were generally positive.

The effects of site-level densities of resident *Trifolium* species ranged from positive to negative, with the strength and direction of effects depending on the resident and focal species (Appendix S1: Tables S2, S3). The strength of individual species interactions did not differ consistently between neighbor removal treatments.

Self-limitation

We assessed the degree of self-limitation for each resident–invader combination by quantifying the relative degree to which site-level density of the resident species influenced its own performance vs. the performance of the invader. Across all species combinations, self-limitation was significantly stronger with neighbors removed than with neighbors present (permutation test, $P < 0.001$); however, most pairwise interactions were neutral (Fig. 2). These results indicate that soil feedbacks and other indirect effects in the absence of neighbors were primarily neutral or stabilizing, whereas feedbacks through direct interactions with neighbors were primarily neutral or destabilizing.

When neighbors were present, we found significant self-limitation for only 1 of 42 resident–invader species combination (Fig. 2), compared to four combinations in which the resident limited the invader significantly more

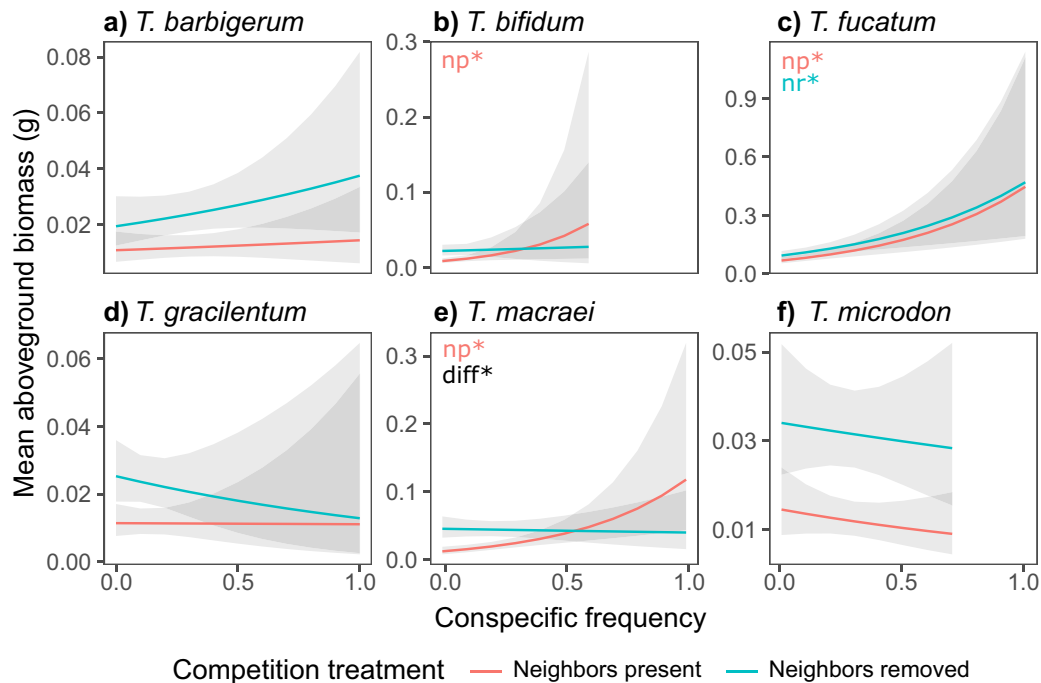


FIG. 1. Effect of conspecific frequency of *Trifolium* species in a field transplant experiment with neighbors present or removed. Colored lines indicate the marginal effect of conspecific frequency for a given focal species and neighborhood treatment. Shaded gray areas indicate 95% confidence intervals. Presence of a statistically significant effect (slope not equal to zero, $\alpha = 0.05$) in the neighbors present (np*) or neighbors absent (nr*) treatments, or of a significant difference in slopes between treatments (diff*), is indicated for each species.

than itself. In contrast, with neighbors removed, we found significant self-limitation for five resident–invader combinations, compared to significant invader-limitation for two combinations (Fig. 2). Out of 15 species pairs for which we could measure self-limitation in both directions, none had significant mutual self-limitation (i.e., both species limited themselves more strongly than the other) in either competition treatment. However, there were some species pairs (one with neighbors present, five with neighbors removed) in which both species had positive, if not statistically significant, self-limitation values, indicating some degree of stabilization.

Self-limitation in natural communities

We assessed self-limitation in natural communities by estimating *Trifolium* species interactions in 265 2-m² plots over two years. We found significant intraspecific competition for four of the six species (*T. barbigerum*, *T. fucatum*, *T. microdon*, and *T. macraei*) for which we could estimate effects (Appendix S1: Table S4). Interspecific interactions ranged from marginally competitive to marginally facilitative, with no significant effect for any species combination (Appendix S1: Table S4). Based on these effects, we found significant self-limitation for 6 of 26 invader-resident combinations and no evidence of significant invader-limitation (Fig. 3). Moreover, out of 13 species pairs for which we could measure self-

limitation in either direction, we found significant mutual self-limitation for two species pairs, and mutually positive if not statistically significant self-limitation values for four additional species pairs. These results indicate stabilizing or neutral interactions for most species combinations in natural communities.

Field and experimental agreement in measures of self-limitation

There was a significant positive correlation between self-limitation in natural communities and self-limitation in the presence of neighbors estimated in our field experiment across species combinations ($R^2 = 0.48$; permutation test $P < 0.001$; Fig. 4). Self-limitation in natural communities was marginally positively correlated with self-limitation in the absence of neighbors ($R^2 = 0.10$; $P = 0.059$; Fig. 4) estimated in the field experiment.

DISCUSSION

A key tenet of modern coexistence theory is that stabilizing niche differences are required for species to coexist stably (Chesson 2000a). We experimentally tested stabilizing forces in the full n -dimensional field niches of eight locally co-occurring *Trifolium* species and assessed the role of plant–neighbor interactions and indirect soil-mediated feedbacks in stabilization. We found evidence

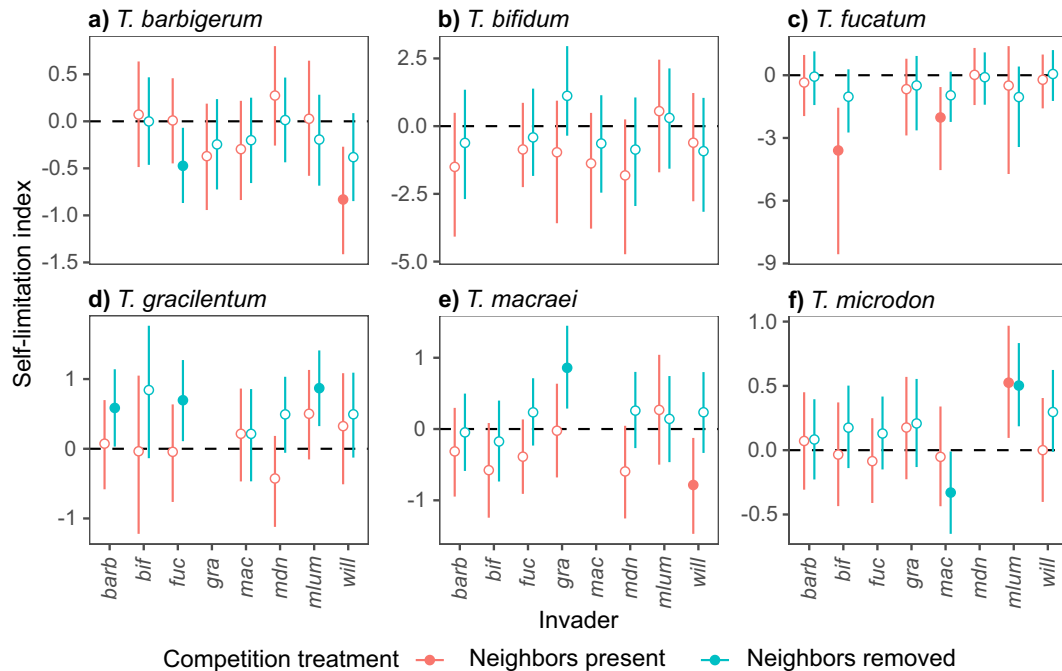


FIG. 2. Self-limitation between *Trifolium* resident–invader combinations measured in a field transplant experiment with neighbors present or removed. Self-limitation is measured as the difference between the resident species' effect on the performance of transplants of the invading species and its effect on the performance of conspecific transplants. Positive values indicate stabilizing self-limitation (resident limits itself more than the invader or facilitates the invader more than itself), and negative values indicate destabilizing invader-limitation (resident limits the invader more strongly than it limits itself or facilitates itself more than the invader). Filled circles indicate statistically significant effects (i.e., bootstrapped 95% confidence interval does not include zero). Invaders are *T. barbigerum* (*barb*), *T. bifidum* (*bif*), *T. fucatum* (*fuc*), *T. gracilentum* (*gra*), *T. macraei* (*mac*), *T. microdon* (*mdn*), *T. microcephalum* (*mlum*), and *T. willdenovii* (*will*).

of stabilization through soil feedbacks for some species combinations, whereas neighborhood interactions were largely destabilizing. In addition, we found evidence that *Trifolium* species partition spatial variation in the environment, which is expected to maintain coexistence at the among-habitat scale due to storage effects but prevent coexistence at the within-habitat scale because high fitness of residents reduces success of invaders.

Spatial niche partitioning

Trifolium species in our field experiment responded differently to spatial variation in the environment, including soil texture, nutrient availability, and grass cover, which provides evidence of spatial niche partitioning. Critically, experimental transplants performed better in sites with environmental conditions similar to those of sites with high resident conspecific densities (i.e., “home” sites), indicating that *Trifolium* species were specialized to fine-scale habitat variation within our study area. These divergent habitat preferences may promote coexistence at the among-patch scale through spatial storage effects (Chesson 2000b, Snyder and Chesson 2004), although we did not measure the strength of potential storage effects in this study. Habitat specialization is also expected to inhibit coexistence at the within-

patch scale by increasing the relative fitness advantage of resident species over invaders, thus increasing the strength of stabilizing niche differences necessary to maintain coexistence within habitats. The distributional and co-occurrence patterns of some of our study species are consistent with strong habitat specialization. For example, *T. fucatum* has co-occurred with congeners at the scale of tens of meters for over a decade, but it rarely co-occurred with congeners within 2-m² plots. Most congeners performed poorly in sites dominated by *T. fucatum* in our field experiment, indicating that stable coexistence at fine scales is unlikely. Future studies integrating measures of stabilization through spatial storage effects and within-habitat mechanisms such as plant–soil feedbacks and competition would provide valuable insights into coexistence across spatial scales.

Our field experiment primarily aimed to measure stabilizing effects at the within-habitat scale. We therefore attempted to statistically remove effects of among-site environmental variation when measuring stabilization, so that our estimates of stabilization primarily reflect soil feedbacks, plant–plant interactions, and other mechanisms operating at the within-habitat scale. However, it is likely that we were unable to entirely remove habitat effects, including effects of unmeasured environmental variation among and within sites and covariation

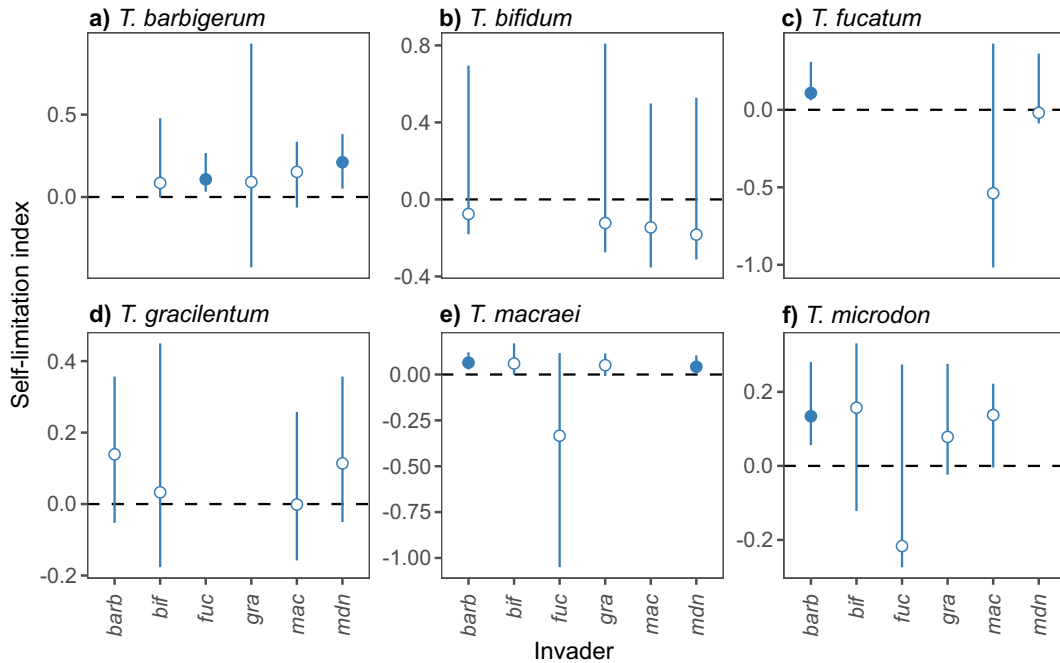


FIG. 3. Self-limitation between *Trifolium* species in natural communities, measured through changes in population densities in field plots ($n = 265$) over two years. Self-limitation is measured as the difference between the resident species' effect on the performance of transplants of the invading species and its effect on the performance of conspecific transplants. Positive values indicate stabilizing self-limitation (resident limits itself more than the invader or facilitates the invader more than itself), and negative values indicate destabilizing invader-limitation (resident limits the invader more strongly than it limits itself or facilitates itself more than the invader). Filled circles indicate statistically significant effects (i.e., bootstrapped 95% confidence interval does not include zero). Invaders are identified in the caption of Fig. 2.

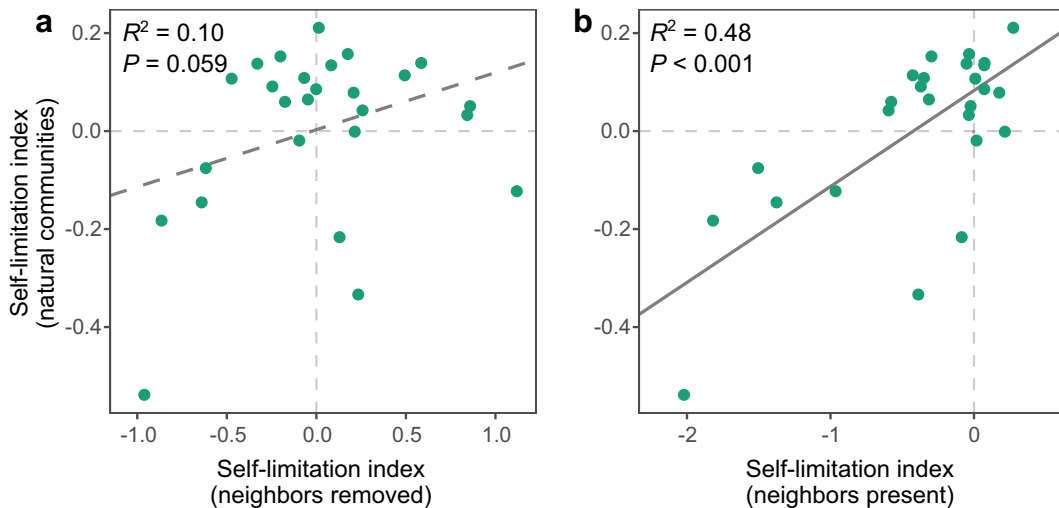


FIG. 4. Relationships between self-limitation of *Trifolium* species estimated from a field transplant experiment with (a) neighbors removed or (b) neighbors present and self-limitation estimated from year-to-year population dynamics in field plots ($n = 265$). Each point represents a *Trifolium* resident–invader pairwise combination for which effects could be measured ($n = 26$). Positive values indicate stabilizing self-limitation (species limits itself more strongly than it limits its congeneric invader), and negative values indicate destabilizing invader-limitation (species limits the invader more strongly than it limits itself).

between environment and resident species densities. This inability may explain the weak stabilization or destabilization we observed for many species pairs in the field

experiment. For example, if the density of a resident species covaried positively with an unmeasured environmental variable, that same variable would also have a

positive effect on conspecific transplant performance in our analysis, which would tend to destabilize coexistence. To the extent that habitat specialization increases the relative fitness advantages of resident species over invaders, our measures of stabilization likely underestimate the strength of stabilizing niche differences in the strict sense of modern coexistence theory, but accurately reflect field dynamics.

Evidence of stabilization through plant-soil feedbacks

We found that high density of *Trifolium* resident species limited growth of conspecifics more strongly than that of congeneric invaders, evidence of stabilizing forces necessary for coexistence, for five of the 42 resident-invader combinations we measured in the competitor removal treatment. For these five species combinations, this finding provides evidence of stabilization in the absence of direct plant-plant interactions, likely through soil-mediated feedbacks and other indirect effects (Bever et al. 1997, Kulmatiski et al. 2008, Crawford et al. 2019). Importantly, our results indicate stabilizing plant-soil feedbacks in realistic field conditions, but the precise mechanisms that led to stabilization are not clear. Possible mechanisms include soil feedbacks mediated by pathogens, mutualists, and abiotic soil properties, as well as mechanisms not related to soil. Accumulation of specialized soil pathogens is the most commonly invoked mechanism of negative plant-soil feedback, and this could explain self-limitation by resident species (Mills and Bever 1998, Bever 2003, Mangan et al. 2010). More recent work suggests that negative feedbacks can occur through mutualism, if the mutualists accumulated by a host species provide greater benefit to heterospecific competitors than to the host itself. Our system is a candidate for such negative mutualist-mediated feedbacks, since *Trifolium* species share mutualists including rhizobia and AMF, and previous greenhouse experiments have shown evidence of negative rhizobially mediated feedbacks in this system (Siefert et al. 2019). There is also the possibility of negative feedbacks mediated by changes in the abiotic characteristics of the soil (Png et al. 2019). Finally, negative net feedbacks could have occurred through mechanisms not related to the soil. For example, it is possible that species experienced greater aboveground herbivory in sites with high conspecific density, though we observed relatively low herbivory across the experiment. Removal of neighbors may also have resulted in changes in microclimate, including increased wind and sun exposure, though it is unclear how these changes may have influenced the strength or direction of frequency dependence.

Destabilization of local coexistence through plant-plant interactions

Our field experiment provided evidence that direct competitive and/or facilitative interactions between *Trifolium* species destabilized coexistence of most species

pairs. Species had more positive frequency dependence and weaker self-limitation when plant neighbors were present compared to when they were absent, indicating that direct plant-plant interactions favored residents over invaders. These results indicate that direct competitive or facilitative interactions between resident species and congeneric invaders should result in the exclusion of the invader in most cases, destabilizing coexistence. Previous work examining the role of plant-plant interactions in stabilizing coexistence has produced mixed results. For example, Adler et al. (2010), working in steppe sagebrush communities, found extremely weak interspecific competition between four shrub species, which, coupled with moderately strong intraspecific competition, resulted in stable coexistence of all species. Similarly, Kraft et al. (2015), working with 18 annual plant species from coastal grassland communities, found stabilizing niche differences, implying stronger intraspecific than interspecific competition, for all species pairs grown in a common garden, although niche differences were not strong enough to overcome relative fitness differences for most species. In contrast, in a meta-analysis of experiments manipulating both plant competition and plant-soil feedbacks, Lekberg et al. (2018) found that interspecific competition was generally stronger than intraspecific competition, indicating that competition often has a destabilizing effect, whereas plant-soil feedback was generally negative and thus stabilizing.

There are several possible explanations for why our results differ from those of previous studies finding that plant competition stabilized coexistence. First, previous studies may have conflated effects of plant neighborhood interactions and plant-soil feedbacks when measuring stabilization, since plant neighbors inevitably alter soil communities. By manipulating the presence of plant neighbors, our study sheds additional light on the relative roles of soil-mediated feedbacks and direct plant-plant interactions in stabilization. Second, our study had a limited phylogenetic scope, including only closely related congeners. Close relatives are likely to have similar resource requirements, potentially leading to strong interspecific competition (Burns and Strauss 2011, Anacker and Strauss 2014) and weak stabilization, though Godoy et al. (2014), working in similar annual plant communities, found no relationship between phylogenetic distance and the strength of stabilizing niche differences. Finally, we note that we only measured species responses to competition in the habitats of their competitors (i.e., invasion into a patch of a resident species). If species experience stronger interspecific competition in away compared to home habitats, our experimental design would overestimate the strength of interspecific competition. Nevertheless, we argue that by examining competitive responses of focal species in the complete niches of residents, our study provides a realistic test of invasibility, a key criterion for stable coexistence (Turelli 1978, Siepielski and McPeck 2010).

Stabilization in natural communities

Estimates of stabilization strength from our field experiment successfully predicted stabilization of population growth rates across *Trifolium* species pairs in natural communities at our field sites, indicating that our experiment captured stabilizing effects that drive coexistence dynamics in nature. Interestingly, our estimates of stabilization with neighbors absent, capturing primarily soil-mediated feedbacks, and stabilization due to neighborhood interactions were both marginally correlated with stabilization of population dynamics in natural communities, indicating that both mechanisms likely contribute to coexistence (or competitive exclusion) in the field.

Although our field experiment successfully predicted the relative strength of stabilization across species pairs in natural communities, it underestimated the strength of stabilization overall. For example, in the experiment we found significant self-limitation for only 1 of 42 species combinations with neighbors present, compared to 6 of 26 species combinations in natural communities. Stabilization in natural communities resulted from a combination of strong negative effects of conspecific density and weak effects of congener density on population growth rates. The fact that we found stronger self-limitation of populations in natural communities than in the field experiment suggests that the experiment underestimated or failed to capture important stabilizing mechanisms operating in nature. For example, because we transplanted plants as seedlings and harvested them prior to setting seed, we may have missed stabilizing mechanisms related to pollination or regeneration niches (Silvertown 2004, Benadi and Pauw 2018).

It is important to note that, while our experiment focused on stabilizing mechanisms, coexistence depends on the relative strength of stabilization and average fitness differences between species. Whether niche differences promote or hinder coexistence also depends on the degree to which they alter relative fitness differences, and recent studies have demonstrated that niche differences that promote stabilization can also drive relative fitness differences (Kandlikar et al. 2019, Song et al. 2019). Because we were not able to measure relative fitness differences in a systematic way in our field experiment or natural communities, we cannot say whether the stabilizing mechanisms we observed were strong enough to overcome relative fitness differences and stabilize coexistence.

CONCLUSIONS

The study of species coexistence rests on a strong theoretical foundation that asserts that stabilizing niche differences, which cause species to limit themselves more than they limit other species, are required for coexistence (Chesson 2000a). Empirical tests of stabilization and the mechanisms behind it are required to link this theory

with coexistence dynamics in nature (Adler et al. 2010, Godoy and Levine 2014, Kraft et al. 2015). Here, we found evidence of stabilizing self-limitation between pairs of locally co-occurring congeneric plant species in natural communities, and we showed that the strength of stabilization between species pairs is explained by the degree of self-limitation through plant–soil feedbacks and direct interactions measured in a field experiment. Notably, we found that plant–soil feedback effects in the absence of neighbors had a stabilizing effect for some species combinations, whereas direct interactions with neighbors were mostly destabilizing. The value of this study in particular is its measurement of stabilizing forces in the full n -dimensional field niche.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.3290/suppinfo>

DATA AVAILABILITY

Data and code are available on Zenodo (Siefert 2020): <https://doi.org/10.5281/zenodo.4290270>