



Contemporary evolution rivals the effects of rhizobium presence on community and ecosystem properties in experimental mesocosms

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Abstract

Because genotypes within a species commonly differ in traits that influence other species, whole communities, or even ecosystem functions, evolutionary change within one key species may affect the community and ecosystem processes. Here we use experimental mesocosms to test how the evolution of reduced cooperation in rhizobium mutualists in response to 20 years of nitrogen fertilization compares to the effects of rhizobium presence on soil nitrogen availability and plant community composition and diversity. The evolution of reduced rhizobium cooperation caused reductions in soil nitrogen, biological nitrogen fixation, and leaf nitrogen concentrations that were as strong as, or even stronger than, experimental rhizobium inoculation (presence/absence) treatments. Effects of both rhizobium evolution and rhizobium inoculation on legume dominance, plant community composition, and plant species diversity were often smaller in magnitude, but suggest that rhizobium evolution can alter the relative abundance of plant functional groups. Our findings indicate that the consequences of rapid microbial evolution for ecosystems and communities can rival the effects resulting from the presence or abundance of keystone mutualists.

Keywords Cooperation · Eco-evolutionary feedback · Legume-rhizobium symbiosis · Mutualism · Rapid evolution

Introduction

Rapid evolution has the potential to alter community and ecosystem processes (Hendry 2020). For example, plant evolutionary responses to reduced herbivory affect decomposition (Fitzpatrick et al. 2015), and guppy evolutionary responses to predation affect stream productivity and nutrient fluxes, as well as invertebrate abundance (Bassar et al. 2010). While many such evolutionary effects on communities and ecosystems have been detected (reviewed in Hairston et al. 2005; Schoener 2011; Hendry 2020), we might assume that the presence or abundance of a species will typically be more important than its evolutionary history and that the effects of evolution may pale in comparison to species presence. However, sometimes that is an erroneous assumption. For example, the effects of *Pseudomonas* local adaptation equaled the effects of *Pseudomonas* presence on microbial community composition (Gomez et al. 2016). Similarly, longitudinal analyses of experimental microcosms suggest that the rapid evolution of algal prey equaled the effects of prey abundance on predator population growth (Becks et al. 2012).

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Such strong effects of evolution might be particularly likely when traits mediating the likelihood or outcome of species interactions evolve and especially in host-microbe resource mutualisms (terHorst and Zee 2016). First, mutualisms are notoriously susceptible to environmental change (Six 2009), and theory predicts strong effects of changing environments on the evolution of resource mutualists (reviewed in Kiers et al. 2010; Bronstein 2015; Porter & Sachs 2020). Given the large population sizes, potential for horizontal gene transfer (e.g., Haskett et al. 2016), and short generation times of microbial mutualists like rhizobia, these evolutionary responses may occur exceptionally rapidly. Second, resource mutualisms play key roles in plant community composition (e.g., Clay and Holah, 1999; van der Heijden et al. 2006; Keller 2014; Kardol et al. 2018) and nutrient cycling (e.g., Vitousek and Walker 1989), and sometimes also affect the abundance of herbivores and higher trophic levels (e.g., Keller et al. 2018). Accordingly, any evolutionary shift in the outcome or strength of mutualism may affect communities and ecosystems.

To go beyond documenting the potential for evolution to affect the ecology and to assess its relative importance compared to traditional ecological factors like species presence or abundance requires experiments that manipulate both species presence and evolutionary history. Here, we tackle this challenge by taking advantage of a long-term experiment that has resulted in the evolution of less cooperative microbial symbionts (Weese et al. 2015). The legume-rhizobium mutualism involves belowground rhizobium bacteria providing their legume hosts with fixed atmospheric nitrogen (N) in exchange for carbon fixed through photosynthesis. This well-studied mutualism is known to respond strongly to N-availability; increased soil N typically reduces the benefits of mutualism for one or both partners (e.g., Arrese-Igor et al. 1997; Unkovich and Pate 1998; Heath and Tiffin 2007; Regus et al. 2017), and theory predicts and empirical work shows that high soil N can cause the evolution of less cooperative rhizobia (West et al. 2002, Akçay and Simms 2011, Weese et al. 2015, but see Simonsen et al. 2015, Wendlandt et al. 2022). Specifically, our previous work capitalized on an N-addition experiment at a Long-Term Ecological Research (LTER) site to show that plants inoculated with rhizobium strains isolated from 20-year-old N-addition plots produced 17–30% less biomass (depending on host plant species) than plants inoculated with rhizobium strains isolated from adjacent control plots (Weese et al. 2015).

Given that the presence of rhizobia can affect both soil N availability (Zahrán 1999) and plant community composition and diversity (Bauer et al. 2012; Keller 2014; Keller and Lau 2018), we hypothesized that the rapid evolution of reduced rhizobium quality would reduce soil N availability and legume dominance, thereby altering plant community composition. We tested this hypothesis by creating

experimental mesocosms that simulate the plant communities found in the N-addition LTER experiment and experimentally manipulating both the presence and evolutionary history of rhizobium symbionts. Because of the previously observed declines in rhizobium quality in the N-addition LTER plots, we predicted that mesocosms inoculated with rhizobium strains isolated from N-addition treatments would have lower biological N-fixation rates, lower concentrations of N (in both soils and plant tissues) and lower biomass of N-fixing clover hosts, but potentially increased biomass of competing functional groups (grasses and non-leguminous forbs), compared to mesocosms inoculated with the rhizobium strains isolated from control plots. Because we also include uninoculated (no rhizobia added) treatments in our experiment, we can compare the magnitude of evolutionary effects to the effects of rhizobium presence in our simulated communities. Our study provides data on how rapid evolution in a key plant-microbe mutualism may influence ecosystem processes and shows that some evolutionary effects are as strong, or even stronger, than rhizobium presence.

Materials and methods

Experimental overview

To test the effects of rhizobium evolution and rhizobium inoculation on plant communities and ecosystem processes (soil N-availability), we created experimental mesocosms simulating the early successional plant communities from which rhizobium populations were originally isolated. We then imposed three rhizobium treatments: uninoculated, inoculated with rhizobium populations that had been evolving in N-addition plots for 20 years, or inoculated with rhizobium populations that had been evolving in adjacent control (no N-added) plots. We measured effects of rhizobium treatments on soil N and plant communities (diversity, productivity, composition) and, for a subset of replicates, we also estimated foliar N concentrations and biological N fixation using the natural abundance $\delta^{15}\text{N}$ method (Kohl et al. 1980).

Rhizobium treatments

Rhizobium strains studied here derive from a long-term N-addition experiment at the Kellogg Biological Station (KBS) LTER site. Briefly, since 1988, this experiment has applied 12.3 g N m^{-2} as ammonium nitrate to 6 replicated $5 \text{ m} \times 5 \text{ m}$ plots in the early successional plant community plots in the LTER main cropping system experiment (MCSE: <http://lter.kbs.msu.edu/research/long-term-experiments/main-cropping-system-experiment/>). An additional unfertilized control plot is adjacent to each N-addition plot. These N-addition treatments have caused dramatic shifts in

plant community composition, including declines in legume abundance (Dickson and Gross 2013).

Three strains isolated from each field plot in 2008 were used in the experiment described herein ($N=3$ strains per plot $\times$ 12 plots = 36 strains). Strains were chosen as a random subset of the ~6 strains per plot characterized by Weese et al. (2015), and all strains successfully nodulate all three *Trifolium* species included in our experiment. Weese et al. (2015) provide a full description of sampling procedures and phenotypic effects of individual strains, while Klinger et al. (2016) provide additional phylogenetic and genomic characterization. Briefly, rhizobia were originally isolated by collecting soil samples from each plot and inoculating *Trifolium pratense*, *T. repens*, and *T. hybridum* seedlings in the greenhouse. These species are the dominant legume species found at the site, are the only legumes that are still reliably found in both N-addition and control field plots, and exhibit similar biomass responses to the rhizobium strains investigated here (Weese et al. 2015). We then collected and surface-sterilized nodules produced by these greenhouse-reared plants and crushed and streaked nodules onto agar plates that were then incubated at 30 °C. Isolates were serially re-plated until single colonies were obtained.

We created experimental mesocosms simulating the early successional communities in the KBS N-addition experiment by planting seedlings of ten species into 15 L pots filled with potting media [60% MetroMix (SunGro Horticulture Canada Ltd., Alberta, Canada), 35% sand, and 5% Turface MVP® (Profile Products LLC, Buffalo Grove, IL)]. Potting media is relatively low in nutrients and was not sterilized prior to planting because previous studies indicated that plants grown in this media did not typically form nodules (Lau and Hammond, personal observation), suggesting that no compatible rhizobia were present in the media. This assumption is further strengthened by observations that responses to rhizobium treatments decline with time and observations that contamination increases over time, suggesting that contamination in our experiments typically results from dispersal into pots post-planting rather than from the initial soil media (see below). Species comprising the experimental mesocosms included ten abundant species found in both the N-addition and control field plots (grasses: *Bromus inermis*, *Dactylis glomerata*, *Phleum pratense*, *Setaria faberi*; legumes: *Trifolium hybridum*, *T. pratense*, *T. repens*; forbs: *Achillea millefolium*, *Daucus carota*, *Hypericum perforatum*). *Trifolium* seeds were obtained from the USDA National Genetic Resources Program (<http://www.ars.grin.gov/>; accession #s: PI 230229, PI 241078, PI 278839), and seeds of all other species were collected from early successional fields adjacent to the N-addition and control plots. Seedlings were originally sown in flats, and then four seedlings of each species were transplanted into each mesocosm, except that two *D. glomerata* seedlings were planted.

We then imposed one of three rhizobium treatments on the experimental mesocosms: uninoculated, rhizobia isolated from N-addition field plots, or rhizobia isolated from control field plots. For mesocosms receiving rhizobia, each mesocosm received a mixture of three strains isolated from a single field plot ($n=12$ different inocula). Each inoculum was applied to 12 replicate mesocosms ($N=12$ inocula $\times$ 12 replicates = 144 plus 36 uninoculated controls, for a total of 180 mesocosms). To create these mixed inocula, single strains were grown in liquid media for 46 h, diluted to a standard optical density ($OD_{620}=0.1$; ca 10^5 cells mL^{-1}), and combined in equal volumes. Next, 0.25 mL of the appropriate mix was applied to each *Trifolium* seedling (3 mL total per mesocosm). To ensure that an adequate number of rhizobia were introduced to the mesocosms, pots were inoculated on two separate occasions: at the start of the experiment and after 38 days.

After 10 weeks of growth and after the *Trifolium* and several other species were in full flower, we harvested above-ground biomass, separated biomass by species, and collected soil samples from each mesocosm with a soil core. Because of the dense root masses after 10 weeks of growth, we were unable to measure belowground traits (e.g., root masses or nodule numbers). To estimate total aboveground productivity and the abundance of each plant species, we weighed biomass samples after drying for at least three days at 65 °C. To estimate soil N availability, we performed KCl extractions on each soil sample and colorimetrically measured ammonium (NH_4^+) and nitrate (NO_3^-) concentrations with an Alpkem/OI Analytic Flow Solution IV analyzer (Model 3550) (Dickson and Gross 2013). We also estimated leaf C and N concentrations of *Trifolium hybridum* and the competing species *Achillea millefolium* for a subset of our experimental mesocosms by grinding a subsample of leaves to a fine powder with a mortar and pestle. *Trifolium hybridum* and *A. millefolium* were chosen for nutrient analysis because they were the most abundant (in terms of biomass) leguminous and non-leguminous forbs in the mesocosms. Analytical duplicates of each sample were analyzed for N and C elemental and isotopic composition on an Elementar Vario Micro Cube elemental analyzer (Hanau, Germany) interfaced to an Isoprime 100 continuous flow isotope ratio mass spectrometer (Cheadle Hulme, UK). The average value for the duplicates was used in data analyses. Reference gases for isotopic analyses were calibrated against USGS40 and USGS41 standards, and NIST 1537 peach leaves were used as standards throughout each sample run. NIST 1570a spinach leaves were used as quality check standards ($n=8$) that had coefficients of variation of 0.9% and 0.8% for N and C concentrations, respectively, and standard deviations of 0.17 ‰ for $\delta^{15}N$ and 0.07 ‰ for $\delta^{13}C$ across all sample runs.

The N isotopic composition of the NH_4^+ and NO_3^- pools in the potting media used in the mesocosms was measured to

characterize the isotopic signature of soil-derived inorganic N. Leaf N isotopic composition results from the mixture of these soil-derived inorganic sources of N and atmospheric N, such that it can be used to approximate the relative contribution of the two sources. N fixation usually leads to a more negative $\delta^{15}\text{N}$ value of plant tissue because the process discriminates against ^{15}N and the N_2 source pool for N fixation has an isotopic signature of 0‰. In cases where the N isotopic composition of the inorganic N pools utilized by the plant is more positive than what is fixed by rhizobia (as might be expected in many natural field soils), the N isotopic composition of the inorganic N pools utilized by the plant is expected to be more positive than what is fixed by rhizobia. As a result, plant uptake of fixed N would lead to a more negative $\delta^{15}\text{N}$ value of plant tissue. However, if as observed in our study, the N isotopic composition of the inorganic N pools utilized by the plant is more negative than what is fixed by rhizobia (See Results), then plant uptake of fixed N would lead to a less negative $\delta^{15}\text{N}$ value of plant tissue. Five replicate subsamples of the potting media were extracted in 2 M KCl. The NH_4^+ and NO_3^- in the soil extracts were separately collected onto Whatman #3 filter paper disks using the acid trap diffusion technique (Herman et al. 1995). The filter paper disks were analyzed using the elemental analyzer and isotope ratio mass spectrometer as described above, except with potassium nitrate and ammonium chloride standards that had been calibrated against the USGS40 and USGS41 standards.

Statistical analyses

We tested separately for the effects of rhizobium evolution and the effects of rhizobium *presence* on (1) soil NH_4^+ concentrations (soil NO_3^- values were extremely low and often below the detection limits of our assay, likely due to our use of low N potting media and the high plant productivity observed in the mesocosms) (2) *Trifolium* leaf N concentration and isotopic composition, (3) *Achillea* leaf N concentration and isotopic composition, (4) total productivity and plant community diversity (Shannon's diversity), and (5) the productivity of plant functional groups with nested MANOVA and ANOVA (Proc GLM and Proc MIXED, SAS Institute 2009). Rhizobium evolution and presence effects were tested separately because of the nested structure of rhizobium evolution treatments (i.e., the unit of replication for rhizobium evolutionary history is inoculum rather than individual mesocosms). We also tested for effects of rhizobium abundance and rhizobium evolution on plant community composition with PERMANOVA ('adonis' function of *vegan* using the Bray–Curtis distance measure with 9999 permutations; 3.0.2, R core development team; Oksanen et al. 2013). In all cases, model assumptions were assessed by visual inspection of residual probability plots and by

testing for homogeneity of variances by comparing models where variances were allowed to vary among rhizobium treatments to those with constant variances. To compare the effects sizes of rhizobium evolution to rhizobium presence/absence treatments, we calculated partial eta-squared values from a series of models comparing each pairwise combination of rhizobium treatments using the effect size option in Proc GLM. The model structures paralleled those presented in the tests of rhizobium abundance effects (e.g., Tables S2, S4, S6, see below), and we used Proc GLM for this calculation so that both rhizobium evolution effects and rhizobium presence effects were calculated based on identical model structures.

Tests of rhizobium evolution effects

Only inoculated mesocosms were used to test for the effects of rhizobium evolution, by including rhizobium evolutionary history (control rhizobia vs. N-addition rhizobia) as a fixed factor. To account for the spatial structure of the original field experiment (one N-addition plot and one control plot within each LTER field replicate), LTER field replicate was included as a random factor. Inoculum (nested within rhizobium evolutionary history and LTER field replicate) also was included as a random factor, and inoculum was specified as the error term for tests of rhizobium and LTER field replicate effects. Harvest date was included as a covariate because it took four weeks to complete the harvest. Because experiments often find that the strongest effects of rhizobia on plant growth are observed at early growth stages, because contamination increases over time in these experiments minimizing treatment differences (Lau and Heath, unpub. data), and because initial analyses revealed interactions between the harvest date covariate and rhizobium treatments, we included interactions between harvest date and fixed effects when they approached statistical significance. When LTER field replicates and harvest date covariates did not approach statistical significance ($P > 0.15$) and removing them improved model fit (or at least did not reduce model fit) based on AIC comparisons, they were removed from analyses to increase power.

Tests of rhizobium presence effects

Uninoculated mesocosms were compared to mesocosms inoculated with rhizobia isolated from control LTER field plots and mesocosms with rhizobia isolated from N-addition LTER field plots to study the effects of rhizobium presence on soil and plant nutrient composition and plant communities. These comparisons were conducted separately because uninoculated mesocosms could not be included in the evolutionary analyses described above because of the nested structure (6 replicate populations per rhizobium evolutionary

history) of the evolutionary analyses. We used MANOVAs consisting of the sets of response variables described above, but only included rhizobium treatment (3 levels: uninoculated vs. inoculated with control rhizobia vs. inoculated with N-addition rhizobia) as a fixed factor and harvest date as a covariate.

Results

Ecological and evolutionary effects of rhizobia on soil nitrogen

Rhizobium evolutionary responses to long-term N-addition affected soil N availability. Mesocosms that were inoculated with rhizobia from N-addition field plots had ~10% less ammonium compared to mesocosms inoculated with rhizobia from control plots (ANOVA NH_4^+ : $F_{1,139} = 6.62$, $P = 0.011$; Fig. 1, Table S1). These effects were strongest for mesocosms that were harvested early (significant Evolutionary history $\times$ Harvest day effects, Table S1). Rhizobium presence had similar effects to rhizobium evolution, with uninoculated mesocosms having 25% less ammonium than mesocosms inoculated with control rhizobia (pairwise contrast $P = 0.022$), although uninoculated mesocosms did not differ significantly from mesocosms inoculated with N-addition rhizobia (pairwise contrast $P = 0.18$). (ANOVA: NH_4^+ : $F_{2,173} = 3.61$, $P = 0.029$, Fig. 1; Table S2). Effect size estimates of rhizobium evolution (partial eta-squared = 0.046)

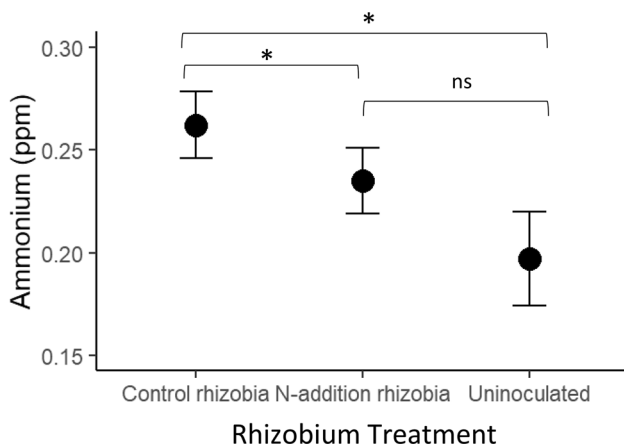


Fig. 1 LS means $\pm$ 1 SE of soil ammonium concentrations (ppm) of mesocosms inoculated with rhizobia from control field plots, mesocosms inoculated with rhizobia from N-addition field plots, or uninoculated mesocosms. Statistical significance of differences between treatments indicated as follows: * $P < 0.05$ and is based on the tests for evolutionary effects (comparisons between Control rhizobia and N-addition rhizobia) and ecological effects (comparisons between: (1) Control rhizobia and uninoculated treatments and (2) N-addition rhizobia and uninoculated treatments) described in the text and presented in Tables S1 and S2, respectively

were small to moderate in magnitude and slightly higher than the effect sizes of models comparing uninoculated mesocosms to mesocosms inoculated with control rhizobia (partial eta-squared 0.02) and much larger than the effect sizes of models comparing uninoculated mesocosms to ones inoculated with N-addition rhizobia (partial eta-squared 0.0003).

Ecological and evolutionary effects of rhizobia on biological nitrogen fixation and foliar nutrients

The observed differences in soil N availability are consistent with differences in biological N fixation. The $\delta^{15}\text{N}$ isotopic signature of the inorganic N pools in the potting soil, 3.77 ‰ for NH_4^+ and -32.28 ‰ for NO_3^- , led to a more negative $\delta^{15}\text{N}$ value of plant tissue than the $\delta^{15}\text{N}$ isotopic signature of N fixed by rhizobia. In such cases, a greater contribution of biological N fixation would cause plant tissue $\delta^{15}\text{N}$ to become less negative, counter to what is commonly observed in field soil. *Trifolium* $\delta^{15}\text{N}$ was highest (least negative) when mesocosms were inoculated with rhizobia isolated from control treatments, suggesting higher biological N fixation (Evolutionary history $F_{1,11} = 5.64$, $P = 0.038$; Fig. 2a, Table S3A). These mesocosms also had higher leaf N concentrations compared to *Trifolium* growing in mesocosms inoculated with rhizobia from N-addition treatments ($F_{1,10} = 6.69$, $P = 0.027$; Fig. 2b; Table S3A). We note, however, that the MANOVA tests of evolutionary history effects on foliar *Trifolium* nitrogen concentrations and isotopic composition were not statistically significant ($P = 0.11$, Table S3A). Uninoculated mesocosms did not differ from mesocosms inoculated with rhizobia from N-addition LTER field plots, but tended to have lower *Trifolium* $\delta^{15}\text{N}$ values than mesocosms inoculated with rhizobia from control LTER field plots ($F_{2,86} = 3.51$, $P = 0.030$; Fig. 2a). *Trifolium* in uninoculated mesocosms did not differ from inoculated mesocosms in foliar N (uninoculated vs. control rhizobia $P = 0.27$; uninoculated vs. N-addition rhizobia $P = 0.29$; the significant rhizobium effect in Table S4A is due to differences between the N-addition rhizobia and control rhizobia treatments). In sum, the effects of rhizobium evolution on *Trifolium* leaf $\delta^{15}\text{N}$ and N concentrations were moderate in magnitude and nearly equal to or even stronger than the effects of rhizobium abundance (partial eta-squared *Trifolium* leaf $\delta^{15}\text{N}$: rhizobium evolution = 0.080, control rhizobia vs. uninoculated = 0.089, N-addition rhizobia vs. uninoculated = 0.001; *Trifolium* leaf percent N: rhizobium evolution = 0.093, control rhizobia vs. uninoculated = 0.023, N-addition rhizobia vs. uninoculated = 0.020).

Leaf $\delta^{15}\text{N}$ in the non-legume competitor *Achillea* was not altered by rhizobium presence or rhizobium evolution (Tables S3B, S4B). However, *Achillea* leaf N was increased slightly in inoculated mesocosms, significantly so for mesocosms inoculated with rhizobium from control LTER field

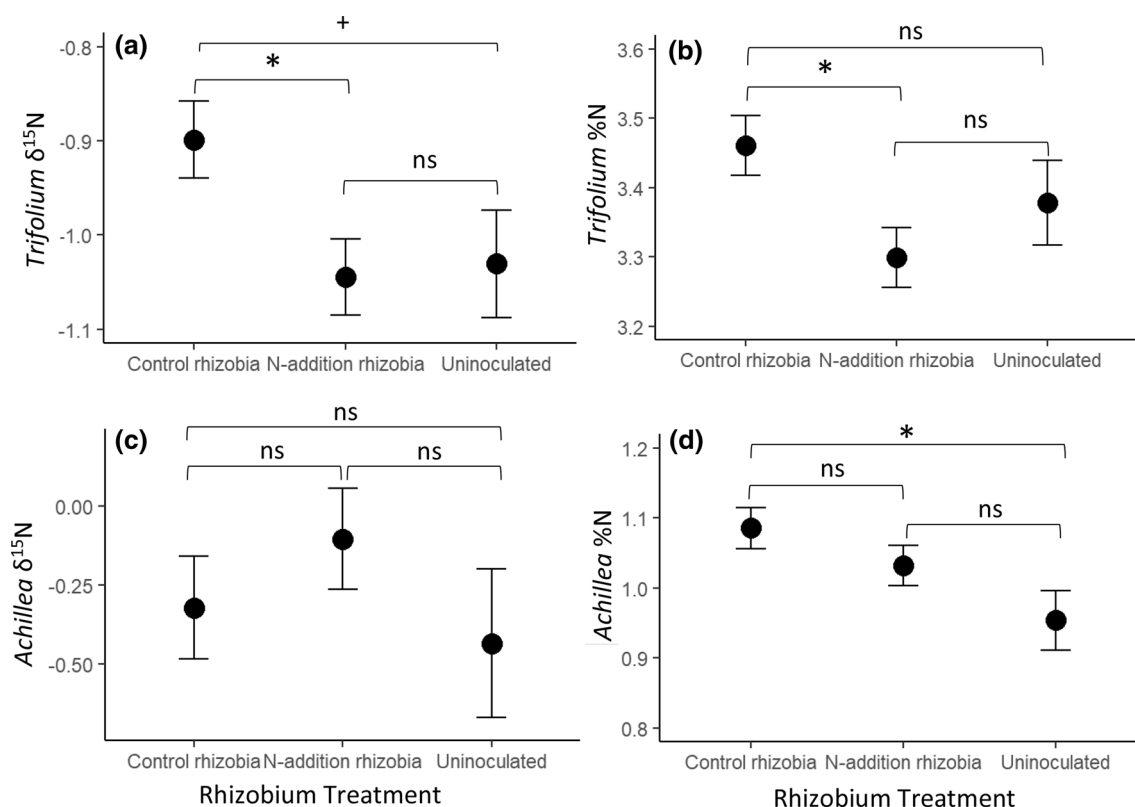


Fig. 2 LS means $\pm$ 1 SE of *Trifolium* $\delta^{15}\text{N}$ (a), *Trifolium* leaf N concentration (b), *Achillea* $\delta^{15}\text{N}$ (c), and *Achillea* leaf N concentration (d) from mesocosms inoculated with rhizobia from control field plots, mesocosms inoculated with rhizobia from N-addition field plots, or uninoculated mesocosms. Statistical significance of differences between treatments is indicated as follows: $^+P < 0.1$, $*P < 0.05$ and is

based on the tests for evolutionary effects (comparisons between Control rhizobia and N-addition rhizobia) and ecological effects (comparisons between: (1) Control rhizobia and uninoculated treatments and (2) N-addition rhizobia and uninoculated treatments) described in the text and presented in Tables S3 and S4, respectively

plots, relative to uninoculated mesocosms ($F_{2,55} = 4.74$, $P = 0.013$; Table S4B; Fig. 2c), potentially indicating reduced competition for soil N. We detected no evidence that rhizobium evolution affected *Achillea* leaf N (Table S3).

Ecological and evolutionary effects of rhizobia on plant communities

We detected little evidence that rhizobium evolution or presence affected plant community composition, in terms of the relative abundance of individual species (perMANOVA rhizobium evolution: $F = 1.46$, $P > 0.20$; abundance: $F = 0.70$, $P > 0.57$). However, we did find evidence that both rhizobium presence and rhizobium evolution affected the abundance of three broad functional groups (legumes, non-leguminous forbs, and grasses) (MANOVA: Rhizobium evolution $F_{3,8} = 4.79$, $P = 0.034$; Rhizobium presence $F_{6,344} = 2.75$, $P = 0.013$; Tables S5, S6). Specifically, the evolution of less-beneficial rhizobia in response to long-term N-addition in the field resulted in increased forb and grass biomass in the mesocosms

(by 27% and 3.9%, respectively), with the greatest effects of evolutionary history on forb biomass observed early in the experiment (Evolutionary history: Forb biomass: $F_{1,109} = 13.09$, $P = 0.0005$; Grass biomass $F_{1,139} = 4.11$, $P = 0.044$; see also Evolutionary history $\times$ harvest day interactions in Table S5; Fig. 3). Rhizobium presence/absence caused similar effects. Forb biomass in uninoculated mesocosms increased by 14.7% when compared to mesocosms inoculated with rhizobium from control plots, but tended to have less forb biomass than mesocosms inoculated with rhizobia from N-addition plots (Forb biomass $F_{2,70} = 7.35$, $P = 0.0013$; Table S6; Fig. 3). As with other metrics, the effects of rhizobium evolution on the biomass of competing functional groups, while small to moderate in magnitude, equaled or exceeded the effects of rhizobium abundance (partial eta-squared grass biomass: rhizobium evolution = 0.029, control rhizobia vs. uninoculated = 0.026, N-addition rhizobia vs. uninoculated = 0.00; forb biomass: rhizobium evolution = 0.089, control rhizobia vs. uninoculated = 0.045, N-addition rhizobia vs. uninoculated = 0.0054).

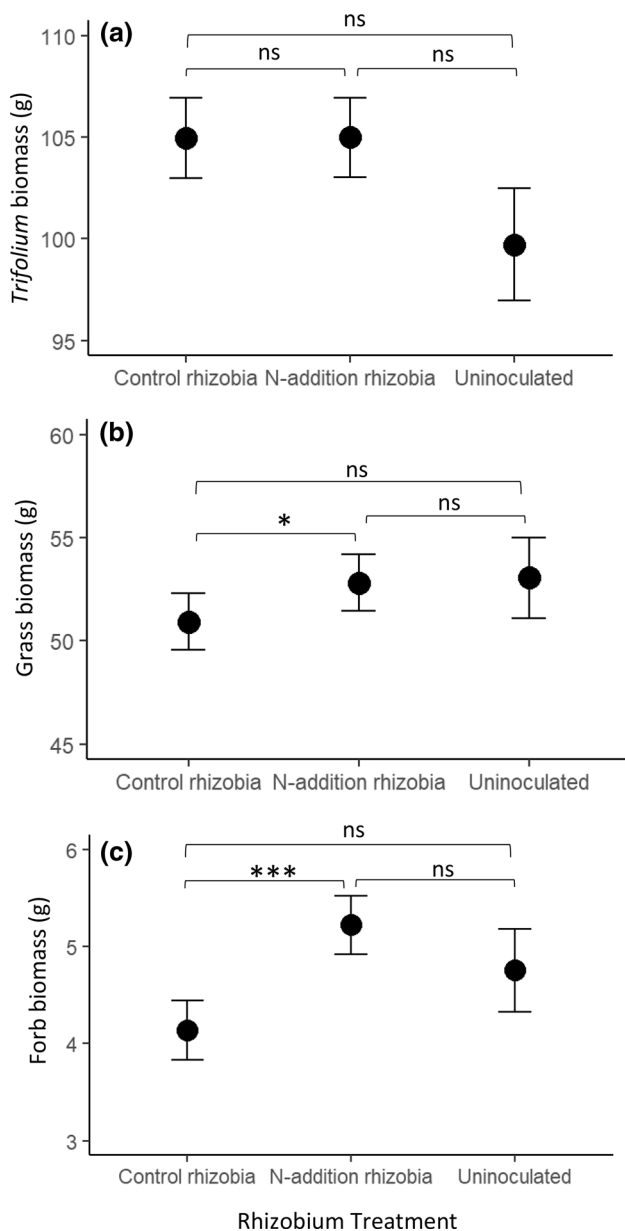


Fig. 3 LS means ± 1 SE of *Trifolium* aboveground biomass (a), grass aboveground biomass (b), and forb aboveground biomass (c) from mesocosms inoculated with rhizobia from control field plots, mesocosms inoculated with rhizobia from N-addition field plots, or uninoculated mesocosms. Statistical significance of differences between treatments is indicated as follows: * $P < 0.05$, *** $P < 0.001$ and is based on the tests for evolutionary effects (comparisons between Control rhizobia and N-addition rhizobia) and ecological effects (comparisons between: (1) Control rhizobia and uninoculated treatments and (2) N-addition rhizobia and uninoculated treatments) described in the text and presented in Tables S5 and S6, respectively

Surprisingly, neither rhizobium evolution nor rhizobium presence affected *Trifolium* biomass (Table S6; Fig. 3). Effects of rhizobium evolution and presence on plant species diversity (Shannon Diversity Index) and productivity

also were small in magnitude (partial eta-squared < 0.025) and were not statistically significant (Tables S7, S8).

Discussion

Over the past decades, numerous studies have illustrated how human-caused environmental changes can elicit evolutionary responses [e.g., biological invasions (Lau 2006) or climate change (Bradshaw and Holzapfel 2006; Schlüter et al. 2014; Schaum et al. 2017) (reviewed in Palumbi 2001; Lau and terHorst 2019)]. Evolutionary responses are even observed over the course of multi-year ecological field experiments (e.g., Snaydon and Davies 1982, Turley et al. 2013, Fitzpatrick et al. 2015, Weese et al. 2015, Nguyen et al. 2016, Magnoli and Lau 2020, reviewed in Strauss et al. 2008). These evolutionary changes, in turn, yield the potential for evolution to influence ecological processes over contemporary time scales (e.g., Palkovacs et al. 2009, Bassar et al. 2010, ter Horst et al. 2014, Fitzpatrick et al. 2015, Declerck et al. 2015, van Diepen et al. 2017; reviewed in Hairston et al. 2005, Schoener 2011, Hendry 2020). Here we demonstrate that the evolution of less cooperative rhizobia in response to 20 years of N-fertilization reduces soil N availability and foliar leaf nitrogen and alters the productivity of plant functional groups. Notably, these evolutionary effects often even equal or exceed the ecological effects of rhizobium presence. Given the key roles that plant–microbe interactions play in both community and ecosystem processes and the evolutionary lability of microbial symbionts, evolutionary shifts in the outcomes of plant–microbe interactions may be both common and ecologically relevant.

Previous work in this system capitalized on a long-term N-addition experiment to test basic theory predicting that increased resource availability causes evolutionary declines in the quality of rhizobium mutualists. The magnitude of evolutionary response was striking, with rhizobia originating from N-addition field plots providing 17–30% less growth benefit to their plant hosts compared to rhizobia originating from nearby unfertilized control plots (Weese et al. 2015). These differences in growth benefits likely reflect differences in N-fixation; plants inoculated with rhizobium strains isolated from N-addition plots also had significantly lower chlorophyll concentrations, an indicator of plant N content (Weese et al. 2015). These previous studies focused on characterizing rhizobium quality and phenotypes under relatively simplistic growing conditions (single strain inoculations on single plants) and on plant individual growth properties. Here, we investigated how rhizobium populations (the 3-strain mixtures used here) influence plant communities and ecosystem processes in more complex simulated early successional plant communities, and also compare the

magnitude of these evolutionary effects to the ecological effects of rhizobium presence.

Rhizobium evolution strongly affected N availability in ways consistent with theory. Evolutionary reductions in rhizobium quality yielded reduced soil ammonium availability compared to the more cooperative rhizobia isolated from control field plots. The observed effects on soil N availability were paralleled by similar trends in biological N fixation and leaf tissue N concentrations in *Trifolium* host plants. Together, these results suggest that the reduced soil N concentrations in mesocosms inoculated with low-quality rhizobia were likely due to increased legume uptake of existing soil N because they were unable to meet their nitrogen needs through biological N fixation. These effects of rhizobium evolution rivaled the ecological effects observed by comparing mesocosms inoculated with rhizobia to uninoculated mesocosms and also are likely to result from different mechanisms—the uninoculated mesocosms likely experienced reduced nodule numbers, while inoculation with the less cooperative strains used here often leads to high nodule numbers, but presumably low N-fixation per nodule (Weese et al. 2015).

Surprisingly the evolution of reduced quality rhizobia did not significantly affect *Trifolium* biomass in these competitive mesocosms. Our previous experiments (e.g., Weese et al. 2015), which were conducted on single plants in pots, showed effects of rhizobia evolution on plant growth that were three- to sixfold greater than those observed here. These contrasting effects suggest that the effects of rhizobium presence and rhizobium evolution may be less apparent in diverse communities, where other ecological interactions may reduce some of the benefits of mutualism. Even inoculation had only minimal and not statistically significant effects (~6% increases) on *Trifolium* abundance. Although other studies conducted in the presence of competitors detect significant and sometimes strong effects of rhizobium presence on host plant growth (e.g., Bauer et al. 2012; Keller 2014; Keller and Lau 2018), experiments manipulating rhizobium presence or genotypes are most often conducted in relatively simplistic growing conditions, so it remains unclear to what extent the community context will alter the fitness outcomes of mutualism. Because our mesocosms had massive root biomass, root binding could potentially reduce growth differences (both *Trifolium* abundance and total community productivity) between treatments given that small pot sizes typically reduce plant growth (e.g., Ray and Sinclair 1998). Furthermore, the dense root systems prevented effective screens for contamination of uninoculated controls, so it is likely that contamination reduced our observed effects on *Trifolium* biomass and also may mean that our rhizobium presence/absence treatments might be more analogous to rhizobium abundance (low

vs. high rhizobium density) treatments. This could also explain why several observed responses were greater for mesocosms harvested earlier since contamination among pots increases with time (Lau and Heath, personal observation). However, the spacing between pots in this experiment exceeded that of our previous single strain inoculation work (e.g., Weese et al. 2015; Heath et al. 2020), making contamination less likely. Furthermore, any contamination likely makes any observed differences among rhizobium treatments conservative, given that contamination should homogenize rhizobium treatments.

Interestingly, even though the little effect on *Trifolium* biomass was detected, mesocosms that were either inoculated with rhizobia from N-addition LTER treatments or left uninoculated resulted in increased grass and non-leguminous forb productivity. These findings corroborate other findings illustrating the community effects of resource mutualists (e.g., Bauer et al. 2012; Keller 2014; Keller and Lau 2018), but also expand on this body of literature to show that evolutionary declines in mutualist quality can cause similar community-level effects to eliminating or reducing the abundance of resource mutualists in the system.

Caveats

The experimental design we employed tested for ecological effects of evolution in low N conditions, where resource mutualisms are predicted to be most beneficial to plant hosts (e.g., Johnson 1997; Schwartz and Hoeksema 1998; Hoeksema and Schwartz 2003; Grman et al. 2012) and where one might expect the effects of rhizobium evolution also to be most apparent. For example, differences in the plant growth benefits of effective and ineffective *Bradyrhizobium* strains on the native annual host plant *Acmispon strigosus* were greatest at low N and eliminated at high N (Regus et al. 2017). We observe similar findings in our system (Lau and Heath, unpublished data). Other studies, however, detect more idiosyncratic responses among both plant and rhizobium genotypes to N, so evidence that low N environments consistently maximize the observed variation in rhizobium quality remains mixed (Heath and Tiffin 2007; Heath et al. 2010). Still, our choice of soil media may have maximized evolutionary effects. Theoretically, it would even be possible that the strains from N-addition plots could provide more growth benefits and fixed N in high N environments compared to control plot strains, though we suspect this is unlikely as additional work suggests that the benefits of N-addition strains never exceed those provided by control strains, even in high N environments (Lau and Heath, unpub. data).

Conclusions and implications for eco-evolutionary feedbacks in plant-microbial systems

We now have a solid appreciation that evolution is frequently rapid and can affect community and ecosystem function (Hendry 2020). What is less clear is when these evolutionary effects are strong enough to rival the effects of the presence or abundance of the focal taxa. Here we show that rhizobium evolutionary responses to long-term nitrogen fertilization frequently equal and sometimes exceed the effects of rhizobium presence on nutrient availability and the abundance of plant functional groups.

Plant-microbe symbiotic systems, like the one studied here, may be strong candidates for important eco-evolutionary feedbacks in natural communities (terHorst and Zee 2016). In many such systems, the microbial player is likely to alter the soil nutrient environment in ways that feedback to influence the outcome of symbiosis and potentially the evolution of the soil microbial symbiont or the plant host. For example, over decadal timescales, invasion by an exotic legume led to less mutualistic soil communities, possibly because increased legume dominance led to soil nitrogen increases and selection favoring less cooperative mutualists (although pathogen accumulation also could not be ruled out) (Lau and Suwa 2016). Increased resource availability does not always lead to the evolution of less cooperative rhizobia (e.g., Simonsen et al. 2015; Wendlandt et al. 2022), however, potentially because in many cases such evolutionary responses to N may be indirect and due to changes in the plant community (reduced host density) rather than direct effects of nitrogen on plant control of the interaction (see Wendlandt et al. 2022). An emerging question is when such evolutionary effects in key microbial symbionts are likely and when such evolutionary effects might be expected to have long-term effects on communities or ecosystem processes.

Our study illustrates the potential for evolution to affect ecosystem processes, but in N-fertilized environments, the contribution of even high-quality rhizobium symbionts to available soil N is likely to be lessened. In other words, when synthetic N-inputs are high, biological nitrogen fixation is likely to be minimal. As a result, any ecosystem-level effects of the evolution of reduced cooperation will most likely be observed once fertilization ceases, and long-term ecosystem effects likely will depend on the extent to which the evolution of reduced cooperation is reversible. Will rhizobium quality and biological nitrogen fixation recover quickly once N-fertilization is ceased, allowing soil N availability to remain high? Or will the evolutionary reductions in rhizobium quality observed in N-fertilized environments persist, yielding lower soil N availability in the absence of further synthetic inputs? Given high levels of standing genetic variation in rhizobium quality in even N-addition populations (Weese et al. 2015), it is possible

that N-addition populations could revert back to high-quality partners rapidly when fertilization is stopped. If genetic variation has been depleted, however, then recovery may be slowed and the evolution of reduced cooperation could leave lasting impacts on these communities. Ultimately, while human-caused environmental changes, like the N-fertilization studied here, may catalyze rapid evolutionary responses to jump start eco-evolutionary feedbacks, the persistence of long-term feedbacks are likely to be determined by a wide variety of factors.

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Author contribution statement JAL, KDH, and DJW conceived the experiments. JAL, DJW, and MDH designed the experiments. DSW, JES, and MDH performed the experiments. WHY conducted the isotope analyses. JAL and DJW analyzed the data. JAL and DJW wrote the bulk of the initial manuscript; WHY wrote the isotope analysis methods; all other authors edited the manuscript.

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Code availability Code used for the data analyses presented in this paper has been made publically available from the Dryad Data Repository (<https://doi.org/10.5061/dryad.cfxpnvx6t>).

Declarations

Conflict of interest Not applicable.

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References

- Akçay E, Simms EL (2011) Negotiation, sanctions, and context dependency in the legume-rhizobium mutualism. *Am Nat* 178:1–14. <https://doi.org/10.1086/659997>
- Arrese-Igor C, Minchin FR, Gordon AJ, Nath AK (1997) Possible causes of the physiological decline in soybean nitrogen fixation

- in the presence of nitrate. *J Exp Bot* 48:905–913. <https://doi.org/10.1093/jxb/48.4.905>
- Bassar RD, Marshall MC, López-Sepulcre A, Zandonà E, Auer SK, Travis J, Pringle CM, Flecker AS, Thomas SA, Fraser DF, Reznick DN (2010) Local adaptation in Trinidadian guppies alters ecosystem processes. *Proc Nat Acad Sci* 107:3616–3621. <https://doi.org/10.1073/pnas.0908023107>
- Bauer JT, Kleczewski NM, Bever JD, Clay K, Reynolds HL (2012) Nitrogen-fixing bacteria, arbuscular mycorrhizal fungi, and the productivity and structure of prairie grassland communities. *Oecologia* 170:1089–1098. <https://doi.org/10.1007/s00442-012-2363-3>
- Becks L, Ellner SP, Jones LE, Hairston NG Jr (2012) The functional genomics of an eco-evolutionary feedback loop: linking gene expression, trait evolution, and community dynamics. *Ecol Lett* 15:492–501. <https://doi.org/10.1111/j.1461-0248.2012.01763.x>
- Bradshaw WE, Holzapfel CM (2006) Evolutionary responses to rapid climate change. *Science* 312:1477–1478. <https://doi.org/10.1126/science.1127000>
- Bronstein JL (2015) Mutualism. Oxford University Press, Oxford. <https://doi.org/10.1093/acprof:oso/9780199675654.001.0001>
- Clay K, Holah J (1999) Fungal endophyte symbiosis and plant diversity in successional fields. *Science* 285:1742–1744. <https://doi.org/10.1126/science.285.5434.1742>
- Declerck SAJ, Malo AR, Diehl S, Waasdorp D, Lemmen KD, Proios K, Papakostas S (2015) Rapid adaptation of herbivore consumers to nutrient limitation: eco-evolutionary feedbacks to population demography and resource control. *Ecol Lett* 18:553–562. <https://doi.org/10.1111/ele.12436>
- Dickson TL, Gross KL (2013) Plant community responses to long-term fertilization: changes in functional group abundance drive changes in species richness. *Oecologia* 173:1513–1520. <https://doi.org/10.1007/s00442-013-2722-8>
- Fitzpatrick CR, Agrawal AA, Basiliko N, Hastings AP, Isaac ME, Preston M, Johnson MTJ (2015) The importance of plant genotype and contemporary evolution for terrestrial ecosystem processes. *Ecology* 96:2632–2642. <https://doi.org/10.1890/14-2333.1>
- Gomez P, Paterson S, De Meester L, Liu X, Lenzi L, Sharma MD, McElroy K, Buckling A (2016) Local adaptation of a bacterium is as important as its presence in structuring a natural microbial community. *Nat Commun* 7:12453. <https://doi.org/10.1038/ncomms12453>
- Grman E, Robinson TMP, Klausmeier CA (2012) Ecological specialization and trade affect the outcome of negotiations in mutualism. *Am Nat* 179:567–581. <https://doi.org/10.1086/665006>
- Hairston NG, Ellner SP, Geber MA, Yoshida T, Fox JA (2005) Rapid evolution and the convergence of ecological and evolutionary time. *Ecol Lett* 8:1114–1127. <https://doi.org/10.1111/j.1461-0248.2005.00812.x>
- Haskett TL, Terpolilli JJ, Bekuma A, O'Hara GW, Sullivan JT, Wang P, Ronson CW, Ransay JP (2016) Assembly and transfer of tripartite integrative and conjugative genetic elements. *Proc Natl Acad Sci USA* 113:12268–12273. <https://doi.org/10.1073/pnas.1613358113>
- Heath KD, Tiffin P (2007) Context dependence in the coevolution of plant and rhizobial mutualists. *Proc R Soc B* 274:1905–1912. <https://doi.org/10.1098/rspb.2007.0495>
- Heath KD, Stock AJ, Stinchcombe JR (2010) Mutualism variation in the nodulation response to nitrate. *J Evol Biol* 23:2494–2500. <https://doi.org/10.1111/j.1420-9101.2010.02092.x>
- Heath KD, Podowski JC, Heniff S, Klinger CR, Burke PV, Weese DJ, Yang WH, Lau JA (2020) Light availability and rhizobium variation interactively mediate the outcomes of legume-rhizobium symbiosis. *Am J Bot* 107:229–238. <https://doi.org/10.1002/ajb2.1435>
- Hendry AP (2020) Eco-evolutionary dynamics. Princeton University Press, New Haven. <https://doi.org/10.1515/9781400883080>
- Herman D, Brooks P, Ashraf M, Azam F, Mulvaney R (1995) Evaluation of methods for nitrogen-15 analysis of inorganic nitrogen in soil extracts. II diffusion methods. *Commun Soil Sci Plant Anal* 26:1675–1685. <https://doi.org/10.1080/00103629509369400>
- Hoeksema JD, Schwartz MW (2003) Expanding comparative-advantage biological market models: contingency of mutualism on partner's resource requirements and acquisition trade-offs. *Proc R Soc B* 270:913–919. <https://doi.org/10.1098/rspb.2002.2312>
- Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phyt* 135:575–586. <https://doi.org/10.1046/j.1469-8137.1997.00729.x>
- Kardol P, De Long JR, Mariotte P (2018) Soil biota as drivers of plant community assembly. In: Ohgushi T, Wurst S, Johnson A (eds) Aboveground–belowground community ecology. Ecological studies (analysis and synthesis), vol 234. Springer, Cham. https://doi.org/10.1007/978-3-319-91614-9_13
- Keller KR (2014) Mutualistic rhizobia reduce plant diversity and alter community composition. *Oecologia* 176:1101–1109. <https://doi.org/10.1007/s00442-014-3089-1>
- Keller KR, Lau JA (2018) When mutualisms matter: rhizobia effects on plant communities depend on host plant population and soil nitrogen availability. *J Ecol* 106:1046–1056. <https://doi.org/10.1111/1365-2745.12938>
- Keller KR, Carabajal S, Navarro F, Lau JA (2018) Effects of multiple mutualists on plants and their associated arthropod communities. *Oecologia* 186:185–194. <https://doi.org/10.1007/s00442-017-3984-3>
- Kiers ET, Palmer TM, Ives AR, Bruno JF, Bronstein JL (2010) Mutualisms in a changing world: an evolutionary perspective. *Ecol Lett* 13:1459–1474. <https://doi.org/10.1111/j.1461-0248.2010.01538.x>
- Klinger CK, Lau JA, Heath KD (2016) Ecological genomics of mutualism decline in nitrogen-fixing bacteria. *Proc R Soc B* 283:20152563. <https://doi.org/10.1098/rspb.2015.2563>
- Kohl DH, Shearer G, Harper JE (1980) Estimates of N₂ fixation based on differences in the natural abundance of ¹⁵N in nodulating and nonnodulating isolines of soybeans. *Plant Phys* 66:61–65. <https://doi.org/10.1104/pp.66.1.61>
- Lau JA (2006) Evolutionary responses of native plants to novel community members. *Evolution* 60:56–63. <https://doi.org/10.1554/05-376.1>
- Lau JA, Suwa T (2016) The changing nature of plant-microbe interactions during a biological invasion. *Biol Invasions* 18:3527–3534. <https://doi.org/10.1007/s10530-016-1245-8>
- Lau JA, terHorst CP (2019) Evolutionary responses to global change in species-rich communities. *Ann New York Acad Sci*. <https://doi.org/10.1111/nyas.14221>
- Magnoli SM, Lau JA (2020) Evolution in novel environments: do restored prairie populations experience strong selection? *Ecology* 101:e03120. <https://doi.org/10.1002/ecy.3120>
- Nguyen MA, Ortega AE, Nguyen KQ, Kimball S, Goulden ML, Funk JL (2016) Evolutionary responses of invasive grass species to variation in precipitation and soil nitrogen. *J Ecol* 104:979–986. <https://doi.org/10.1111/1365-2745.12582>
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) vegan: community ecology package. R Package version 2.0-10 <http://cran.r-project.org/package=vegan>
- Palkovacs EP, Marshall MC, Lamphere BA, Lynch BR, Weese DJ, Fraser DF, Reznick DN, Pringle CM, Kinnison MT (2009) Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Phil Proc R Soc B* 364:1617–1628. <https://doi.org/10.1098/rstb.2009.0016>
- Palumbi SR (2001) Humans as the world's greatest evolutionary force. *Science* 293:1786–1790. <https://doi.org/10.1126/science.293.5536.1786>

- Porter SS, Sachs JL (2020) Agriculture and the disruption of plant-microbial symbiosis. *Trends Ecol Evol* 35:426–439. <https://doi.org/10.1016/j.tree.2020.01.006>
- Ray JD, Sinclair TR (1998) The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. *J Exp Bot* 49:1381–1386. <https://doi.org/10.1093/jxb/49.325.1381>
- Regus JU, Wendlandt CE, Bantay RM, Gano-Cohen KA, Gleason NJ, Hollowell AC, O'Neill MR, Shahin KK, Sachs JL (2017) Nitrogen deposition decreases the benefits of symbiosis in a native legume. *Plant Soil* 414:159–170. <https://doi.org/10.1007/s11104-016-3114-8>
- Schaum C-E, Barton S, Bestion E, Buckling A, Garcia-Carreras B, Lopez P, Lowe C, Pawar S, Smirnov N, Trimmer M, Yvon-Durocher G (2017) Adaptation of phytoplankton to a decade of experimental warming linked to increased photosynthesis. *Nat Ecol Evol* 1:0094. <https://doi.org/10.1038/s41559-017-0094>
- Schlüter L, Lohbeck KT, Gutowska MA, Gröger JP, Riebesell U, Reusch TBH (2014) Adaptation of a globally important coccolithophore to ocean warming and acidification. *Nat Clim Change* 4:1024–1030. <https://doi.org/10.1038/nclimate2379>
- Schoener TW (2011) The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. *Science* 331:426–429. <https://doi.org/10.1126/science.1193954>
- Schwartz MD, Hoeksema JD (1998) Specialization and resource trade: biological markets as a model of mutualisms. *Ecology* 79:1029–1038. [https://doi.org/10.1890/0012-9658\(1998\)079\[1029:SARTBM\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1998)079[1029:SARTBM]2.0.CO;2)
- Simonsen AK, Han S, Rentschler CS, Heath KD, Stinchcombe JR (2015) Short-term fertilizer application alters phenotypic traits of symbiotic nitrogen fixing bacteria. *PeerJ* 8:e1291. <https://doi.org/10.7717/peerj.1291>
- Six DL (2009) Climate change and mutualism. *Nat Rev Microbiol* 7:686. <https://doi.org/10.1038/nrmicro2232>
- Snaydon RW, Davies TM (1982) Rapid divergence of plant populations in response to recent changes in soil conditions. *Evolution* 36:289–297. <https://doi.org/10.1111/j.1558-5646.1982.tb05044.x>
- Strauss SY, Lau JA, Schoener TW, Tiffin P (2008) Evolution in ecological field experiments: implications for effect size. *Ecol Lett* 11:199–207. <https://doi.org/10.1111/j.1461-0248.2007.01128.x>
- ter Horst CP, Zee PC (2016) Eco-evolutionary dynamics in plant-soil feedbacks. *Funct Ecol* 30:1062–1072. <https://doi.org/10.1111/1365-2435.12671>
- ter Horst CP, Lennon JT, Lau JA (2014) The relative importance of rapid evolution for plant-soil feedbacks depends on ecological context. *Proc R Soc B*. <https://doi.org/10.1098/rspb.2014.0028>
- Turley NE, Odell WC, Schaefer H, Everwand G, Crawley MJ, Johnson MTJ (2013) Contemporary evolution of plant growth rate following experimental removal of herbivores. *Am Nat* 181:S21–S34. <https://doi.org/10.1086/668075>
- Unkovich MJ, Pate JS (1998) Symbiotic effectiveness and tolerance to early season nitrate in indigenous populations of subterranean clover rhizobia from S.W. Australian Pastures. *Soil Biol Biochem* 30:1435–1443. [https://doi.org/10.1016/S0038-0717\(97\)00258-7](https://doi.org/10.1016/S0038-0717(97)00258-7)
- van der Heijden MGA, Streitwolf-Engel R, Riedl R, Siegrist S, Neu-decker A, Ineichen K, Bollner T, Wiemken A, Sanders IR (2006) The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phyt* 172:739–752. <https://doi.org/10.1111/j.1469-8137.2006.01862.x>
- van Diepen LTA, Frey SD, Landis EA, Morrison EW, Pringle A (2017) Fungi exposed to chronic nitrogen enrichment are less able to decay leaf litter. *Ecology* 98:5–11. <https://doi.org/10.1002/ecy.1635>
- Vitousek PM, Walker LR (1989) Biological invasion by *Myrica faya* in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. *Ecol Monogr* 59:247–265. <https://doi.org/10.2307/1942601>
- Weese DJ, Heath KD, Dentinger BTM, Lau JA (2015) Long-term nitrogen addition causes the evolution of less-cooperative mutualists. *Evolution* 69:631–642. <https://doi.org/10.1111/evo.12594>
- Wendlandt CE, Gano-Cohen KA, Stokes PJN, Jonnala BNR, Zomorrodian AJ, Al-Moussawi K, Sachs JL (2022) Wild legumes maintain beneficial soil rhizobia populations despite decades of nitrogen deposition. *Oecologia* 198:419–430. <https://doi.org/10.1007/s00442-022-05116-9>
- West SA, Kiers ET, Simms EL, Denison RF (2002) Sanctions and mutualism stability: why do rhizobia fix nitrogen? *Proc R Soc B* 269:685–694. <https://doi.org/10.1098/rspb.2001.1878>
- Zahrn HH (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol Mol Biol Rev* 63:968–989. <https://doi.org/10.1128/MMBR.63.4.968-989.1999>

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