Resource limitation influences all levels of biological organization, from the vast community of detritivores and saprophytes below-ground, to the primary producers, herbivores, and predators above-ground. In addition to underlying major theories in community ecology (Leibig, 1840; Tilman, 1977, 1985; Bloom et al., 1985), the concept of limiting resources underlies much theory in mutualism ecology and evolution (reviewed by Bronstein, 2015). Symbioses that are based on the exchange of resources are beneficial when they alter patterns of resource limitation in ways that increase the fitness of both partners. Heuristic theory (Collins Johnson, 1993; Bronstein, 1994; Collins Johnson et al., 1997; Kiers et al., 2002; O’Brien et al., 2018), mathematical theory (Schwartz and Hoeksema, 1998; Bever, 2015; Christian and Bever, 2018; Clark et al., 2019), and empirical observations (Collins Johnson et al., 2010, 2015; Zheng et al., 2015;
Ji and Bever, 2016; Shantz et al., 2016; Ossler and Heath, 2018) all indicate that the ecological outcome of resource mutualisms can shift along the mutualism–parasitism continuum depending on the availability of traded resources. Theory also suggests that resource availability will also influence the evolution of resource mutualisms (West et al., 2002; Thrall et al., 2007; Akçay and Simms, 2011). However, our ability to predict mutualism evolution, and the response of mutualisms to environmental change (Six, 2009; Kiers et al., 2010; Shantz et al., 2016), requires a nuanced understanding of how the resource environment alters both the ecological outcomes of mutualism and the quality of different partner genotypes and the expression of genetic variation for mutualism traits.

The legume–rhizobium symbiosis is a classic resource mutualism, wherein the bacteria housed in legume root nodules fix atmospheric dinitrogen (N₂) into plant-available forms and receive fixed carbon (C) generated by photosynthesis in return. As predicted by resource mutualism theory (Schwartz and Hoeksema, 1998; West et al., 2002; Neuhauser and Fargione, 2004; Akçay and Simms, 2011), the fitness outcomes of legume–rhizobium mutualism are known to be sensitive to the external availability of both N (Heath et al., 2010; Barrett et al., 2012; Weese et al., 2015; Regus et al., 2017; Forrester and Ashman, 2018) and C (light) (Sprent, 1973; Murphy, 1986; Hansen et al., 1990; Myster, 2006; Lau et al., 2012; Ballhorn et al., 2016; Taylor and Menge, 2018). First, increased N typically reduces the plant benefit from associating with rhizobia (Regus et al., 2017; Wendlandt et al., 2019), and plants often (but not always) respond by reducing resource allocation to rhizobia (Streeter and Wong, 1988; Heath et al., 2010; Simonsen et al., 2015; Regus et al., 2017; Wendlandt et al., 2019). Second, the net benefits for plant hosts (i.e., growth or fitness increase from associating with rhizobia, which will be a function of the growth benefits resulting from N gained versus the fitness costs of C spent) are expected to decrease as C becomes more limiting relative to N, since mutualism with rhizobia requires that plants possess adequate C stocks to support these costly N-fixing symbionts (Minchin and Witty, 2005; Pringle, 2016). Thus, low light and high N environments are both predicted to reduce mutualism benefits to both plants and rhizobia (Collins Johnson et al., 1997).

Not all rhizobium mutualists, however, are equally beneficial. Rhizobium strains are well known to vary in partner quality, which is most often measured as relative plant growth and fitness (reviewed by Denison, 2000; Simms and Taylor, 2002; Heath and Stinchcombe, 2014; Sachs et al., 2018). These growth and fitness benefits to the plant depend on both the benefits and costs of symbiosis, i.e., the benefits of fixed N received minus the C costs including nodule formation, nodule respiration, and production of the bacterial storage compound poly-3-hydroxybutyrate (PHB) (Tjepkema and Winship, 1980; Minchin and Witty, 2005; Ratcliffe et al., 2008; Ruess et al., 2013). Plant nutrient status and light interactively determine plant growth (reviewed by Elser et al., 2010); therefore, genetic variation in N-fixing rhizobium symbionts may interact with light availability to influence the outcome of mutualism for plant hosts. Likewise, the relative partner quality of different nutritional symbionts like rhizobia should depend on the resource stoichiometry of the hosts, and thus we might expect that the relative fitness benefits of interacting with different rhizobium inocula might shift as hosts encounter different light environments. For example, variation in rhizobium partner quality might be reduced in low light environments if plants are so C-limited that additional N provides little growth benefit.

Thus, on the one hand, a plant’s ability to respond to a favorable shift in the resource environment, like increased light availability, might depend on its limitation by other resources (like N) and, therefore, the quality of its mutualist partner. On the other hand, the relative quality of different mutualist partners might depend on the resource environment. Here we investigate how the net growth benefits to plants from rhizobia respond to light availability and vary across substantial rhizobium genetic variation. We also measure plant traits associated with the benefits they receive from rhizobia through N fixation (foliar N, C:N ratio, and δ¹⁵N), and with some of the costs associated with nodulation (node number and per-node weight) to better understand how light availability interacts with rhizobium strain variation to shift the costs and benefits of symbiosis. We grew Trifolium hybridum L. (alsike clover; Fabaceae) hosts with one of 11 strains of Rhizobium leguminosarum (Frank, 1889; Skerman et al., 1980; family Rhizobiaceae) in either ambient light or shade in the greenhouse to ask how rhizobium strain and the light environment interact to affect plant growth. These experiments shed light on how genetic variation in rhizobium mutualists mediates the response of plant hosts to different light environments, and reciprocally how the light environment alters the relative benefits of different rhizobia or the expression of rhizobium partner quality variation.

MATERIALS AND METHODS

Study system

We studied the effects of 11 R. leguminosarum (hereafter rhizobium) strains on T. hybridum growth in two different greenhouse light treatments (ambient or shade). These strains were a subset of those studied previously (Weese et al., 2015), and full methods for rhizobium strain isolations and partner quality assessments may be found there. Previously assessed partner quality of the strains may also be found in Appendix S1. Briefly, rhizobia isolated from soils at the Kellogg Biological Station Long Term Ecological Research Site (KBS LTER; http://lter.kbs.msu.edu/) by isolating them from nodules of three Trifolium species (T. hybridum, T. repens, and T. pratense) in a large common garden experiment (Weese et al., 2015). Subsequently, a single-strain common garden experiment (Weese et al., 2015) was used to assess the effects of individual strains on plant growth and chlorophyll content (a proxy for plant N status; Swiader and Moore, 2002). The 11 strains used as inoculum in the current experiment were selected to represent a range of partner quality (see Appendix S1).

Greenhouse experiment

To study how rhizobium genetic variation influences plant responses to light, T. hybridum plants were grown with one of 12 rhizobium treatments (11 strains plus an uninoculated control) in either ambient light or under 50% shade cloth (open on sides to minimize effects on humidity). The split-plot design included two light treatments (ambient or shade) that were applied to whole plots (2 plots per light treatment), and the 12 inoculation treatments were randomly assigned to individual plants within each plot (10 replicates per inoculation treatment per plot; for 480 plants total). We purchased T. hybridum seeds from a local seed supply company (Illini FS, Urbana, IL, USA). Seeds were...
washed extensively and then surface-sterilized for 1 min in ethanol, followed by 10 min in a 5–6% sodium hypochlorite solution before planting into 107 mL SC7 Cone-tainers (Steuewe and Sons, Tangent, OR, USA) containing root wash mix (1:1:1 soil–calcinated clay–tordedo sand). Plants were inoculated at 8 d post-planting with the appropriate rhizobium strain (OD600 = 0.1 or ~10^6 cells). Plants were grown under 14-h days in the greenhouse, provided with supplemental light to reach a maximum 600 W/m^2, given adequate water throughout the experiment, and fertilized with N-free Fahraeus solution (Somasegaran and Hoben, 1994) every 4 d.

Data

At harvest, we gathered data on three main types of symbiotically relevant phenotypes: (1) Data on aboveground and belowground biomass and root–shoot ratio provide information on plant growth responses (i.e., the net benefit of associating with rhizobia), (2) nodule number and nodule mass (mean individual mass of a nodule) as a proxy for host costs of nodulation, allowing us to calculate how the net benefits to plants per infection (per nodule) change across treatment combinations, and finally (3) plant foliar C and N data (C, N, C:N ratio, and δ15N), which provide more direct information on how the balance of C and N shifts across strain and light treatments. Together these growth and functional phenotypes provide a more mechanistic understanding of how rhizobium partner quality affects plant nutrient status and in turn mediates the response of plants to light availability, though we note that some costs and benefits of the symbiosis were not measured directly (e.g., nodule respiration, N acquired from symbiosis, PHB production, any non-C costs of nodulation).

At week 7, 48 plants (one from each plot from each treatment combination) were randomly selected and harvested early for preliminary analysis and to ensure that plants were nodulated. At week 9, we counted the number of leaflets for all remaining plants. The remainder of the experiment was harvested at week 15. At harvest, above- and belowground plant tissue were separated and, for half of the plants in each treatment combination (5 replicates per plot), nodule number was counted and 10 haphazardly chosen nodules were removed, dried at 60°C, and weighed to estimate mean per-nodule mass for each plant (hereafter, nodule mass). Plant tissue was dried at 60°C for at least 48 h before weighing. We calculated per-nodule plant biomass for each plant in the experiment as belowground biomass + aboveground biomass and divided by the total number of nodules on the root system. We calculated root to shoot ratio for each plant by dividing belowground biomass by aboveground biomass.

After harvest, dried leaf tissue from the subset of five replicate plants per treatment and block combination used to estimate nodule number and nodule biomass was submitted to the University of Wyoming’s Stable Isotope Facility (Laramie, WY, USA) for grinding and estimation of C and N content as well as δ15N using a Costech 4010 elemental analyzer coupled to a Thermo Delta Plus XP IRMS (Thermo Fisher, Waltham, MA, USA). Without nonsymbiotic controls, it is not possible to say with certainty how much plant δ15N was derived from symbiotic N fixation (Shearer and Kohl, 1986); therefore, all variation in δ15N levels is relative to other treatment combinations. Because plant N derived from symbiotic N fixation is more similar to atmospheric N in isotope composition (versus soil N), field-grown plants with higher rates of N fixation generally have decreased δ15N values, relative to those with lower fixation rates (Shearer and Kohl, 1986; Handley and Raven, 1992), though these dynamics are more difficult to predict in pot experiments where many drivers of soil N isotope ratios from the field (Craine et al., 2015) may be missing.

While plants were initially inoculated with isogenic populations of a single strain, the fact that all uninoculated plants formed nodules revealed cross-contamination, which generally occurs when bacteria move among neighboring pots (K. D. Heath, personal observation). Control plants had 50% fewer nodules, compared to inoculated plants (72.7 vs. 139.5 nodules, respectively; p = 0.0098). Given the randomized experimental design, this cross-contamination was random with respect to treatment and thus should reduce the likelihood of detecting treatment effects, making tests for genetic differences conservative. The highly significant variation among the 11 strain treatments for all measured variables (see Results; Table 1) indicated that these treatments differed even in the face of contamination. A cautious interpretation, therefore, is that plants in different inoculation treatments formed symbiosis with genetically distinct, but not necessarily isogenic, populations of rhizobia. Uninoculated plants were not included in further analyses.

Analyses

All analyses were implemented in SAS (version 9.2, SAS Institute, Cary, NC, USA). Phenotypic correlations (calculated using PROC CORR) among all measured variables, in both ambient and shade environments, are presented in Appendix S2. We used mixed model ANOVA (PROC MIXED) specifying the Satterthwaite approximation for the denominator degrees of freedom (DDFM=SATTERTHWAIT) to test for the fixed effects of light treatment, rhizobium strain (11 strains), light × strain interaction, and blocking variables (random effect of greenhouse plot nested within light treatment and fixed effect of early vs. late harvest date) on measures of plant growth and nodulation. Random effects were tested using the log-likelihood ratio of nested models as described elsewhere in detail (Littell et al., 1996; Heath, 2010). Because we were interested in proportional rather than absolute changes in most traits across treatment combinations, variables were natural log-transformed before analysis (Wootton, 1994; Hamback and Beckerman, 2003), with the exception of foliar %C and %N (arc sine square-root transformation) and δ15N (not transformed). Qualitatively, results did not depend on the choice of data transformation. In addition, we used separate MANOVA of plant growth traits (early leaflets, aboveground biomass, belowground biomass, root–shoot ratio, per-nodule plant biomass) and foliar nutrient traits (%C, %N, C:N ratio, and δ15N) to test for the overall effects of experimental treatments on these suites of traits.

To investigate how changes in plant biomass were related to nodulation traits and foliar nutrient levels, we calculated correlations between all measured traits using Pearson correlations (PROC CORR) of inoculum trait means (11 inocula in each of two light environments). We used Spearman rank correlations between the 11 inoculum means in ambient versus shade environments to test whether significant interactions of light treatment and rhizobium inoculum (see Results) were driven by changes in rank versus changes in variance. Finally, to explore whether the variation
in plant growth caused by rhizobium inocula of varying quality was magnified in the ambient light environment, we used Levene's tests for homogeneity of variances (implemented in PROC GLM) to test whether the among-inoculum variance in traits differed between light environments.

**RESULTS**

MANOVA indicated strong effects of all model terms on plant growth traits (Table 1A). With few exceptions, the effects of genetically variable rhizobium inocula greatly exceeded the effects of light on plant growth, nodulation, and foliar C and N (Table 1A–C; Fig. 1). For example, inoculation with the highest quality rhizobium strain resulted in ~15x more aboveground biomass on average, compared to the lowest quality strain (493: 1.33 ± 0.49 g versus 498: 0.09 ± 0.04 g). For comparison, plants in the ambient light treatment produced just ~1.3x more aboveground biomass than plants in the shade treatment. However, we also detected evidence that the response of plant hosts to the light environment depended on rhizobium inoculum (significant light × inoculum interactions, Table 1A–C). Plants inoculated with some strains had large biomass increases in ambient light compared to shade (e.g., strain 262: 78% and over 300% for above- and belowground biomass, respectively). In contrast, plants inoculated with other strains did not respond much to increased light availability or even had slightly decreased growth in ambient light (see reaction norms in Fig. 1A, B; Appendix S3). Compared to the interactive effects with rhizobium inoculum, the main effect of shade on plant traits was less dramatic, with marginal reductions in belowground biomass, significant reductions in root–shoot ratio, per-nodule plant biomass, and C:N ratio (34%, 33%, and 23% decrease in shade, respectively; Table 1A, C). We included harvest date as a blocking factor, and its significant effect on nearly all traits was consistent with plants harvested later being larger (e.g., significant effects on biomass and nodule number; Table 1A–C).

As for plant growth, the MANOVA for foliar nutrients indicated strong effects of all model terms on plant growth traits (Table 1C). Percentage N and C, C:N ratio, and δ15N varied widely among inocula (Table 1C; Fig. 1), although the magnitude of the observed strain differences in δ15N varied across light environments (significant inoculum × light interaction on δ15N; Table 1C). Moreover genetic correlations indicate that N content and δ15N strongly predicted aboveground biomass in both light environments (Table 2), which together suggest that the availability of fixed N increased plant biomass. For example, plants inoculated with strains 498 and 699 had extremely high C:N ratios and large, positive δ15N values, suggesting little biologically fixed N in both light environments (Figure 1E, F). These plants made little biomass even in the ambient light environment (Figure 1A, B). On the other hand, inocula generating the most negative δ15N values (e.g., 209, 627), suggesting more biologically fixed N, resulted in large gains in plant biomass when light became less limiting.

Overall, we found a trade-off between nodule number and nodule mass, i.e., inocula producing more nodules tended to produce smaller nodules (Table 2). Unlike plant growth, nodule number and

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**TABLE 1.** Mixed model ANOVA and MANOVA for the effects of light treatment (ambient or shade), rhizobium strain, the light × strain interaction, and blocking variables (models in A include harvest date) on hybrid clover traits, nodule traits, and foliar nutrient composition. For fixed effects, F is shown; for random effects, χ^2^ (log likelihood ratio) is shown. All variables are fixed in MANOVA.

| A. Plant traits | N df | Aboveground biomass | Belowground biomass | Plant biomass per nodule | Root:shoot ratio | Leaflet no. (wk. 9) | MANOVA num df / denom df | MANOVA Wilk’s lambda |
|-----------------|------|---------------------|---------------------|-------------------------|-----------------|---------------------|--------------------------|-----------------------|
| Light           | 1    | 4.39                | 16.69*              | 3.94*                   | 60.95****       | 1.92                | 4 / 279                  | 53.74****              |
| Strain          | 10   | 87.83****           | 55.66****           | 6.92****                | 85.32****       | 40 / 1059.8         | 13.24****                | 627                    |
| Light × Strain  | 10   | 3.00**              | 6.28****            | 0.77                    | 1.68*           | 4.82****            | 40 / 1059.8              | 12.61****              |
| Harvest date    | 1    | 313.44****          | 205.17****          | 13.61****               | 5.30*           | 4 / 279             | 75.69****                |                       |

**Random effects**

Plot (Light)

| 10.2*** | 9.7*** | 0 | 4.0* | 48.1**** | 8 / 558 | 3.15** |

| B. Nodule traits | N df | Nodule no. | Nodule mass |
|------------------|------|------------|-------------|
| Light            | 1    | 251        | 0.3         |
| Strain           | 10   | 18.21****  | 11.04****   |
| Light × Strain   | 10   | 0.42       | 0.37        |
| Harvest date     | 1    | 27.91****  | 2.29        |

**Random effects**

Plot (Light)

| 0 | 3.9* |

| C. Foliar nutrients | N df | Foliar % C | Foliar % N | Foliar C:N ratio | Foliar δ15N | MANOVA num df / denom df | MANOVA Wilk’s lambda |
|---------------------|------|------------|------------|------------------|-------------|--------------------------|-----------------------|
| Light               | 1    | 8.26       | 6.06       | 52.59****        | 0.35        | 4 / 187                  | 21.56****              |
| Strain              | 10   | 5.77****   | 62.67****  | 88.62****        | 66.23****   | 40 / 710.94              | 21.07****              |
| Light × Strain      | 10   | 1.46       | 0.59       | 0.32             | 2.55**      | 40 / 710.94              | 2.84****               |

**Random effects**

Plot (Light)

| 8.0** | 15.9*** | 14.5**** | 4.5* | 8 / 374 | 5.44**** |

Notes: Mixed model denominator degrees of freedom were 282 and 293 for plant biomass per nodule and nodule number (respectively) or between 1.92 and 3.99 (all other variables) for the effect of light, and ranged from 175-405 for other fixed effects. +P ≤ 0.1; *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001.
FIGURE 1. Reaction norms of hybrid clover growth, nodule number, and foliar nutrient composition across two light treatments in symbiosis with 11 N-fixing rhizobium inocula. Raw means are shown.
belowground ground

Plant biomass

Table 2. Genotypic correlations among all dependent variables in ambient light (above the diagonal) or shade (below the diagonal) treatments. Pearson correlation coefficients (N = 11) are shown, with significant correlations (p < 0.05) indicated in bold.

| Variable                      | Root-shoot | Root–shoot Nodule mass | Leaflet no. | Nodule no. | Nodule mass | Foliar % C | Foliar % N | Foliar C:N ratio | Foliar δ15N |
|-------------------------------|------------|-----------------------|-------------|------------|-------------|------------|------------|------------------|------------|
| Leaflet no.                   | 0.974      | 0.774                 | 0.056       | 0.595      | 0.705       | 0.808      | 0.740      | 0.572            | 0.445      |
| Aboveground biomass           | 0.344      | 0.392                 | 0.059       | 0.461      | 0.614       | 0.707      | 0.641      | 0.572            | 0.445      |
| Belowground biomass           | 0.327      | 0.354                 | 0.058       | 0.508      | 0.678       | 0.767      | 0.687      | 0.572            | 0.445      |
| Plant biomass per nodule      | 0.472      | 0.561                 | 0.107       | 0.540      | 0.759       | 0.808      | 0.740      | 0.572            | 0.445      |
| Nodule no.                    | 0.015      | 0.025                 | 0.010       | 0.010      | 0.010       | 0.010      | 0.010      | 0.010            | 0.010      |
| Nodule mass                   | 0.056      | 0.036                 | 0.010       | 0.010      | 0.010       | 0.010      | 0.010      | 0.010            | 0.010      |
| Foliar % C                    | 0.098      | 0.098                 | 0.098       | 0.098      | 0.098       | 0.098      | 0.098      | 0.098            | 0.098      |
| Foliar % N                    | 0.098      | 0.098                 | 0.098       | 0.098      | 0.098       | 0.098      | 0.098      | 0.098            | 0.098      |
| Foliar C:N ratio              | 0.098      | 0.098                 | 0.098       | 0.098      | 0.098       | 0.098      | 0.098      | 0.098            | 0.098      |
| Foliar δ15N                   | 0.098      | 0.098                 | 0.098       | 0.098      | 0.098       | 0.098      | 0.098      | 0.098            | 0.098      |

nodule mass differed among inocula but did not respond to light (no significant effects of light or light × inoculum interactions; Table 1; Fig. 1C). However, the relationship between these nodulation traits (number and mass) and plant growth did depend on the light environment. In ambient light, neither nodule number nor nodule mass predicted shoot biomass (Table 2). In the shade, however, inocula producing abundant nodules resulted in host plants with fewer leaflets and less above- and belowground biomass (Table 2), suggesting the formation of numerous nodules was costly in low light environments. Indeed plant biomass expressed on a per-nodule basis decreased by 34% on average in the shade and depended on inoculum (Table 1; Fig. 1D). Moreover per-nodule plant biomass was positively correlated with nodule size and negatively correlated with both C:N ratio and δ15N in the shade (Table 2), suggesting that inocula that produce fewer, larger nodules were more beneficial for shaded hosts.

Together, our trait data suggest that one inoculum (strain 262) was particularly interesting in the context of net nodulation benefits. Inoculation with 262 resulted in plants that had negative δ15N values and low C:N ratios, similar to other highly beneficial inocula (Fig. 1E, F), yet produced only moderate per-nodule biomass and responded with very large increases in both nodulation and plant biomass in ambient light (Fig. 1A–D). Together these observations suggest that, unlike low-fixing, low-biomass inoculum treatments (strains 498 and 699), an inoculum dominated by strain 262 might result in a high-benefit, high cost symbiosis—fixing adequate N, but not requiring abundant plant C.

While the net growth effects of different rhizobium inocula changed across light environments (light × inoculum interactions; Table 1), this interaction was largely driven by changes in variance rather than rank shifts among different inocula. Spearman rank correlations indicated that the highest quality inocula in ambient light environments were also the most beneficial in low light environments (e.g., early leaflet count, r11 = 0.95, p < 0.0001; aboveground biomass, r11 = 0.85, p = 0.0010; belowground biomass, r11 = 0.83, p = 0.0017; C:N ratio, r11 = 0.59, p = 0.0560; %N, r11 = 0.89, p = 0.0002; δ15N, r11 = 0.71, p = 0.0146). Larger variance among inocula in ambient light for all biomass traits, combined with significant Levene's tests for early leaflet count (F1,20 = 6.98, p = 0.0156), further indicate that the expression of genetic variation in rhizobium quality was magnified when light was more available.

**DISCUSSION**

Our results indicate that (1) variation in rhizobium partner quality is substantial and can mediate plant responses to the light environment, and reciprocally, (2) variation in rhizobium partner quality depends on the light environment. Plant biomass responses, together with data on nodule number and nodule mass as well as foliar C:N ratios and δ15N, suggest that these findings are underpinned by variation in both C costs and N benefits among different rhizobium strains.

**Rhizobium variation mediates host plant responses to light**

The effects of rhizobium strain on plant growth in our experiment were large and dwarfed the main effects of the light environment (see Results), though we note that plants in nature likely have more access to soil N, and there we might expect that rhizobia would have weaker effects compared to light limitation or other environmental...
Ecological and evolutionary effects of light on the legume–rhizobium mutualism

The importance of the light environment on the ecology and evolution of plant–symbiont resource mutualisms has not received much theoretical attention, despite the fact that light controls the availability of an essential traded commodity (plant C). In contrast to other recent studies (Lau et al., 2012; Taylor and Menge, 2018), shaded plants did not significantly reduce allocation to rhizobia (i.e., no significant effects of light or light × inoculum interactions on nodule number or nodule mass), though the observed trends (≈20% reductions in nodule number and nodule mass in shade) were consistent with previous findings. In addition, our light reduction was less severe (50% here, compared to 80% for Lau et al., 2012 and 92% for Taylor and Menge, 2018), and our split-plot design resulted in less power to detect light main effects.

We do find that ambient light environments tend to increase the magnitude of variation among rhizobium inocula, in terms of plant growth (though not nodule traits). This represents a genetic extension of resource mutualism theory showing that the costs and benefits of mutualism change depending on the external availability of traded resources such as C, N, and P (Collins Johnson et al., 1997, 2010; Schwartz and Hoeksema, 1998; Neuhauser and Fargione, 2004). In our study, rhizobium inocula did not change rank across light environments, suggesting that selection on plants to interact with different strains would not depend on the light environment. In contrast, in the mycorrhizal mutualism, decreasing light availability through shading has been shown to alter the relative allocation to different fungal species on host roots (Zheng et al., 2015; Knecht et al., 2016).

Nevertheless our findings suggest that the light environment could be just as important to rhizobium evolution as the more commonly studied N availability (Akçay and Simms, 2011; Regus et al., 2014, 2017; Weese et al., 2015; Klinger et al., 2016). Environmental dependence of variation in rhizobium partner quality might suggest that the plant-mediated feedbacks that select for increased rhizobium partner quality (Kiers et al., 2003; Simms et al., 2006; Heath and Tiffn, 2009; Oono et al., 2011; Regus et al., 2014; Batstone et al., 2017) should be strongest in high light situations, as should selection on plants to evolve such mechanisms (Foster and Kokko, 2006; Heath and Stinchcombe, 2014; Steidinger and Bever, 2014; Bever, 2015; Christian and Bever, 2018). Additional experiments will be necessary to test these hypotheses.

Batstone et al. (2020, in this issue) found that the expression of plant genetic variation for nodule number depended on the environment, whereas we find that rhizobium variation contributing to plant growth benefits differed across light environments. Thus, while we arrive at similar broad-scale conclusions about the importance of context-dependent genetic variation to mutualism evolution, the particulars of which partner (host vs. symbiont) and traits were different. More studies quantifying genetic variation in mutualism traits across environments will be required before we arrive at a predictive synthesis for which traits and environmental variables are likely the most important for context-dependent evolutionary outcomes.

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DATA AVAILABILITY
All data presented in this study are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.hx3flbg9s (Heath et al., 2019).

SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Partner quality information for rhizobium strains used.

APPENDIX S2. Table of phenotypic trait correlations.

APPENDIX S3. Reaction norm plot for total plant biomass.

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