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2016   2018

Long-term eco-evolutionary impacts of the mycorrhiza on plant population dynamics

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Symbioses have played important roles in the evolution and diversification of life. The fitness impacts of symbioses are generally thought to be positive, but actually measured impacts range from extremely positive to extremely negative, depending on partnered species and experimental conditions. Evolutionary studies focused on symbioses assume that the most important impacts will be immediately translated into higher reproduction, resulting in an observable gain or loss in fitness in the short-term. However, symbioses may have extremely variable impacts in the short-term, depending on environmental conditions, identities of symbionts, and the genetics of the interacting species. I used an experimental in situ approach with a wild orchid species in Estonia to address this issue. I found that, indeed, symbioses have variable impacts over the short-term in the wild, but over the long-term they result in greater overall fitness, suggesting a purely adaptive context to their evolution.

Symbioses have profound fitness impacts on species. However, such impacts have generally been inferred in controlled, laboratory or greenhouse conditions, rather than in wild populations. The adaptive value of symbioses should depend not simply on short-term growth or reproduction, but on their direct and indirect impacts on long-term population dynamics. Temporal environmental variation may change the evolutionary contexts of fitness components such that short-term estimates no longer reflect the long-term outcome [1]. This may particularly be the case in long-lived organisms, for which the relationship between fitness and fitness proxies such as growth and fecundity is both complex and non-additive.

In this project, I hypothesized that symbioses reduce the variance in fitness if they affect response to environmental stress. Temporally fluctuating environmental stress results in periods of lower fitness, which in some cases are exacerbated by high growth and resource mobilization during less stressful periods [2], and so increase the long-term variance in fitness. Because such stressful periods have disproportionately large impacts on long-term fitness, symbioses that increase the ability of organisms to handle environmental stress may be adaptive if they stabilize fitness over the long-term, even if short-term fitness is reduced. In the case of the mycorrhiza, an extremely common nutritional symbiosis between most plants and some fungi, this may particularly occur if the mycorrhiza allows plants to expand their nutritional harvest via fungal hyphal growth and nutritional interactions with other plants via plant-soil feedbacks or mycorrhizal networks [3,4].

The potentially variance-reducing impacts of symbioses should be particularly beneficial to long-lived organisms. Fitness is most sensitive to changes in adult mortality rather than reproduction in long-lived organisms. Long-lived herbaceous perennials have a number of strategies for dealing spatiotemporal environmental variability, including vegetative dormancy, in which the plant does not sprout aboveground tissue for potentially years at a time. Intriguingly, this stress response is often linked to the mycorrhiza, because in many herbaceous plants it is a mycotrophic life history stage. Alternatively, growth is also a means of dealing with temporal environmental stochasticity. As plants grow bigger, they grow larger root systems, thereby increasing their nutrient supply and reducing the temporal variance in nutrient uptake by spreading across a larger portion of the surrounding environment. If clonal, plants spread ramets that act as bases for more root growth and foraging through the landscape [5]. Increased size also allows plants to reduce mortality by conveying an increased ability to survive herbivory, competition, and other stressful interactions. Increased size also leads to increased reproduction [6]. Recent evidence suggests that shrinkage may be just as adaptive as growth, and plants may fine-tune their size to changing optima across time [7].

In this project, the symbiosis I focused on is the orchid mycorrhiza. The mycorrhiza is the primary means by which ~ 80-90% of all plant species acquire their mineral nutrition. Unlike most other plants, orchids are obligately dependent on their mycorrhiza. They begin life as dust seeds with little or no energy reserves, during which time virtually their entire carbon budget comes from their mycorrhizal fungi at least until they form mature, photosynthetic shoots [8]. In the wild, orchids cannot recruit to maturity without an appropriate fungal host. Many photosynthetic orchids derive at least some of their carbon in maturity from their mycorrhizal fungi, and some rely exclusively on it [9]. Regardless of type, the mycorrhiza has strong impacts on plant growth that suggest increased plant fitness via the mycorrhiza. It does this primarily by increasing the size and nutrient foraging ability of the root system. The mycorrhiza also influences plant populations via plant-microbial feedback, in which the spread of mycorrhizal fungi and other microbes to nearby plants causes the colonization of those plants by those microbes, and via mycorrhizal networks, in which plant demography is influenced by nutrient flow ultimately derived from other plants via mycorrhizal fungi.

Experiments have demonstrated the adaptive importance of symbioses in the short-term. However, their impacts over the long-term are not well-understood, particularly under natural levels of environmental variation. The long-term impacts of symbioses likely vary with time, space, and population density, and so are almost certainly complex. I tested the long-term impacts of the mycorrhiza on the fitness and population dynamics of an obligately mycorrhizal plant in the wild, and further explored the potentially role of density on these patterns. Through this, I gained an understanding of a) how symbioses affect environmental variability in

fitness and fitness components, b) how fitness varies over time under different levels of symbiotic integration, and c) how population dynamics govern the fitness impacts of symbioses in the wild.

This study tests the long-term fitness impacts of the mycorrhiza on a long-lived orchid species, Epipactis atrorubens, under density-independent and dependent scenarios. It includes:
a) An initial manipulation of plant mycorrhizal systems, followed by long-term monitoring.
b) Laboratory analysis of soil and root samples to determine any impacts of treatment of treatment on soil quality and mycorrhizal colonization.
Deterministic and stochastic (density-independent) demographic analyses, as well as density-dependent transient analysis and prospective simulations, were conducted to assess the full fitness context of the interaction.

This project was begun in 2012 at six field sites in Estonia. The study species is Epipactis atrorubens (Hoffm.) Bess., an obligately mycorrhizal, photosynthetic orchid that grows in calcareous soils in European meadows and forests. This plant is rhizomatous, with roots that live ≥3 yrs. It begins life as a germinated seed that grows quickly into a protocorm, which is a unique, mycotrophic life stage in orchids. The protocorm develops into a micro-rhizome and begins producing roots, typically by Autumn of the same year in which germination occurs. The experiment consists of 'cohorts' of 40 mature plants included in the experiment in each year at each site, with each cohort consisting of four treatment groups of 10 plants each. These are not cohorts in the sense of individuals born at the same time, but adults added into the project at the same time. I added cohorts in a staggered fashion across years to control the experiment for transient year effects, and to keep precision high as individuals from preceding cohorts die off. The four treatment groups included: 1) 5um trenching, in which plants are separated from mycorrhizal networks in situ with Nitex mesh with a 5um pore size, which is small enough to prevent penetration of fungal hyphae without acting as a barrier to nutrient flow, 2) 50um trenching, in which plants are separated from mycorrhizal networks in situ 50um mesh, which has pores large enough to allow fungal hyphae to regrow, 3) replanting, in which plants are physically dug up as in the preceding two treatments but placed back without any mesh, and 4) control, in which plants are used as observational controls and are not dug up or treated in any way. The size of the Nitex mesh is a 40cm × 40cm square, which is large enough to hold the root system and a fair amount of soil in place. E. atrorubens has short, thick roots that do not branch and have no root hairs, so this treatment does not impede root growth. The locations of these individuals within the population were mapped. I began creating these cohorts in July 2012, and repeated this every year thereafter, also boosting the number of sites from four to six.

I conducted chemical analyses on the soil collected from a subset of representative plants to determine shifts in concentrations of NO_3, NH_4, total plant available phosphorus, total magnesium, and total potassium linked to mesh treatment, per established protocols (Turner and Romero 2009). Additionally, I collected root samples from a subset of living individuals at the end of the experiment and assessed mycorrhizal colonization on both a root-length and root cross-section basis.

I based all demographic analysis on a 16 stage life cycle with three juvenile stages (Seed, Protocorm, and Seedling) and 13 adult size classes (from 0 to ≥ 12 leaves). Survival, sprouting, growth, flowering, and fruiting rates/probabilities were estimated via mixed models against size, recent flowering history, treatment, year, and site. The resulting models were used to create function-based matrix models for population projection analyses. Fecundity, germination, growth from protocorm to seedling, and growth from seedling to mature stage X functions were derived from these data and from the published literature. Initial analyses and hypothesis tests included life table response experiments (LTRE) and stochastic life table response experiments (sLTREs). The hypothesis that the mycorrhiza exerts a strongly adaptive influence on plant stability should yield the result that plants treated with 5um mesh would exhibit a strong decline in fitness relative to all other plants, where fitness is estimated as the deterministic population growth rate associated with that treatment group in the LTRE. If there is a long-term adaptive impact of mycorrhizal connection, then this same pattern should occur in the stochastic population growth rate in sLTRE analysis, as well. In both cases, and particularly once I have six years of data, I also tested for the impacts of treatment on specific vital rates, both by exploring the impacts of treatment on mixed models, and by exploring the contributions of shifts in the mean and variance of matrix elements in sLTRE analysis.

To assess the role of the mycorrhiza in density-dependent population dynamics, I developed mixed models predicting survival, sprouting, growth, flowering, and fruiting as functions of treatment, local density, previous size, recent flowering history, year, and site. I tested for transient dynamics in these data, particularly testing the reactivity and resilience of demographic patterns across time. Finally, I used the results of these analyses to create density-dependent population simulations setting density-dependence to levels and kinds observed via mixed models, and varying the levels of frequency of stress in the environment. The main hypotheses were tested via comparisons between the 5um mesh treated plants and the 50um mesh treated plants. However, the other treatments utilized in this experiment allowed me to test for the impacts of other aspects of the trenching. The direct effect of mesh treatment on plant demography was tested via sLTRE analysis of the no-mesh vs. the 50um mesh treatments, and the effect of temporary severing of mycorrhizal connections via digging will be assessed via sLTRE analysis of observational controls vs. no-mesh plants. Comparison of cohorts allowed me to distinguish the impact of treatment from extrinsically-imposed temporal variation in vital rates, particularly via cross-cohort comparisons in transient analysis (Caswell 2001). Finally, shifts in soil mineral concentrations were estimated as the change in concentration of each nutrient between the initial sample taken when a plant was first included and the sample taken three years later.

All treatments led to declines in performance initially, with the biggest declines in the 5um mesh (from lowest to highest vital rates: 5um < 50um < dug up without mesh < not dug up; Fig. 1). We created population projection matrices averaging demographic vital rates across sites and treatments. These matrices reflected transitions among stages as noted before, but without protocorms (which were incorporated into the seedling class), extra-large plants (which we have few of in the previous dataset), and dormant plants (which can only be observed with ≥3 yrs of data).

Deterministic growth rate among treatments varied between 0.7 (5um) and 1.4 (observational controls). LTRE analysis suggested that 5um mesh treated plants exhibited a higher population growth rate than 50um mesh-treated plants (0.82 vs. 0.70 for 5um vs. 50um plants, respectively). This is not consistent with H_1, but is consistent with H_2 because it suggests that temporal variance in population growth rare may be higher in the 5um treatment. Further, treatment impacted growth rate primarily via survival, consistent with H_3, the survival-growth hypothesis (Fig. 1).

Total soil phosphorus after one year was not impacted by mesh (0.046 ± 0.004 vs. 0.053 ± 0.0006 % total soil phosphorus content, 5um vs. 50um, mean ± 1 se). Power analysis suggested that incorporating ≥30 individuals per treatment was enough to observe significant treatment differences in population growth (Fig. 7 left). This number is far smaller than the 380 individuals per treatment we will have for our test of main treatment effects, suggesting high power in our analysis. Further, with 100 individuals per treatment, about half of demographic transitions that varied among matrices did so significantly.
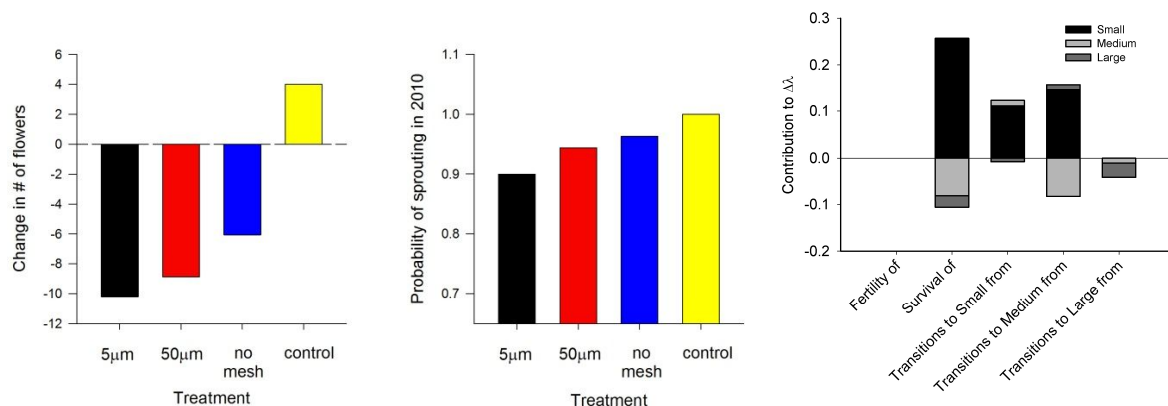

Fig. 1. Impacts of treatment on no. of flowers (left) and probability of sprouting in 2010 (middle) in *E. atrorubens*. LTRE of 5μm treatment with the 50μm treatment as reference (right).

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