NOTE

Soil Microbiomes Underlie Population Persistence of an Endangered Plant Species

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ABSTRACT: Microbiomes can dramatically alter individual plant performance, yet how these effects influence higher-order processes is not well resolved. In particular, little is known about how microbiome effects on individual plants alter plant population dynamics, a question critical to imperiled species conservation. Here we integrate bioassays, multidecadal demographic data, and integral projection modeling to determine how the presence of the natural soil microbiome underlies plant population dynamics. Simulations indicated that the presence of soil microbiomes boosted population growth rates (λ) of the endangered *Hypericum cumulicola* by 13% on average, the difference between population growth versus decline in 76% of patches. The greatest benefit (47% increase in λ) occurred in low-nutrient, high-elevation habitats, suggesting that the soil microbiome may help expand *H. cumulicola*'s distribution to include these stressful habitats. Our results demonstrate that soil microbiomes can significantly affect plant population growth and persistence and support the incorporation of soil microbiomes into conservation planning.

Keywords: integral projection modeling, plant-microbe interactions, endangered species, Florida rosemary scrub, *Hypericum cumulicola*, demography.

Introduction

Microbiomes are ubiquitous in nature and play an outsized role in the performance of their plant and animal hosts (Wardle et al. 2004; Harris 2009). However, ecologists are still in the early stages of scaling these microbiome effects to higher-order ecological processes (Bever et al. 1997; Kardol et al. 2006; Schnitzer et al. 2011). One substantial knowledge gap is how microbiomes affect their host's population dynamics (Ehrlén et al. 2016). Filling this gap requires unification of host-microbiome interactions and demographic modeling, two broad and rapidly growing fields that have largely developed separately from one another (e.g., Ehrlén et al. 2016; Fierer 2017). Better integration of these fields will connect individual-level fitness effects through population dynamics to community patterns and ecosystem processes. In the cases of imperiled species, such integration will additionally inform their management and conservation.

There are two major challenges to linking plant-microbiome interactions to plant population dynamics. First, the magnitude and direction of microbial effects on plant performance often vary across the plant's life stages (Kardol et al. 2013). Second, these stage-specific microbial effects on individuals must be appropriately integrated into the host plant's demography to determine their effects at the population level (e.g., Chung et al. 2015). Therefore, scaling microbial effects to the population level requires both detailed knowledge of plant species' demography and quantification of microbial effects on the critical vital rates (e.g., germination, growth, reproduction) underlying demography. To date, such work has been confined to a single genus of tightly coevolved, endophytic fungi (Yule et al. 2013; Chung et al. 2015) or, in studies that do consider whole-soil microbiomes (e.g., Bever et al. 1997), has largely ignored the importance of demography in population persistence.

Here we investigated the role of soil microbiomes in plant population growth. First, we conducted a bioassay to quantify microbial effects on two critical vital rates of the endangered plant *Hypericum cumulicola*—germination and early plant growth (Picó et al. 2003)—in soils spanning several environmental gradients (e.g., fire history, elevation above the water table). Second, we scaled these individual-level performance effects to population-level consequences using an integral

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projection model (IPM) constructed from our bioassay data and >20 years of field observations from >10,000 individual plants. Our results provide the first evidence that plant population persistence can depend on the soil microbiome, especially in stressful habitats.

Methods

Study System

The Florida scrub ecosystem is highly imperiled, with <15% of its original extent remaining (Weekley et al. 2008). Within this ecosystem, the fire-dependent rosemary scrub habitat occurs at relatively high elevations above the water table (mean = 1.27 ± 0.52 m [SD]; Quintana-Ascencio et al. 2018). *Hypericum cumulicola* (Small) P. Adams, a federally and state-listed endangered species, specializes on open sand gaps found between shrubs in rosemary scrub patches (Quintana-Ascencio et al. 2018). This perennial herb typically germinates in the winter, reproduces between June and September, and dies back in November–December. Individuals are killed by fire, but seeds can survive fire and persist in a long-lived seed bank (Quintana-Ascencio et al. 2018). Previous work indicated that germination of *H. cumulicola* increased with the presence of microbially active soil crusts (Hawkes 2004), suggesting that this species was suitable for our study.

Bioassay

Experimental Design. We grew *H. cumulicola* in a factorial experiment that manipulated the presence of microbes using a soil sterilization treatment ("live" vs. "sterilized" conditions) in soils collected from 14 rosemary scrub patches at Archbold Biological Station, Venus, Florida (27°11′N, 81°21′W). Patches were selected to represent a range of environmental conditions and included six long-term *H. cumulicola* monitoring patches (table A1; tables A1–A4 are available online). In total, the bioassay included 5,880 seeds sown in 196 pots (14 soils × 2 soil sterilization treatments × 7 replicates, with 30 seeds per pot).

Collection of Soils and Seeds. Soils were collected from the interior of open sand gaps at least 1 m from the nearest shrub to a depth of 15 cm. In the field, we sieved soils through a 0.64-cm mesh to remove large debris. Seeds were collected from mature fruits of hundreds of plants (~2 or 3 fruits per plant) growing throughout the northern half of Archbold Biological Station in September 2016. We sorted and removed damaged seeds in the laboratory.

Experimental Setup. To manipulate the presence of soil microbes, we inoculated pots with live (unmanipulated, microbially active soil) or sterilized soils. Pots were filled with a 50-mL base of sterilized soil, a 9-mL inoculation layer (15% soil volume) of live or sterilized soil, and a 2-mL cap of sterilized soil to prevent microbial desiccation. All pots (66 mL; Cone-tainers; Stuewe and Sons, Tangent, OR) and sterilized soils were autoclaved at 121°C twice prior to use, and each pot contained soil from a single rosemary scrub patch. We sowed 30 seeds per pot, which is consistent with previous estimates of seed density.

Data Collection. Seeds were allowed to germinate in a grow room at the University of Miami at room temperature under ambient natural light conditions, adding supplemental fluorescent light at 20 weeks (16L : 8D conditions). We recorded germinants and harvested plants after 8 months to record growth (aboveground height and dried biomass) when plants were comparable to "yearlings" in the field (new, <15-cm-tall, single-stem plants).

We analyzed soil properties associated with the 14 rosemary scrub soils used in the bioassay (assessed by the University of Florida Analytical Research Laboratory, Gainesville, FL). Total C and N were measured using a vario MAX cube CNS analyzer (Elementar Americas, Mount Laurel, NJ), and total P and K were measured using a SPECTRO ARCOS Inductively Coupled Plasma Spectrophotometer (SPECTRO Analytical Instruments, Mahwah, NJ).

We obtained additional patch-specific environmental data for the 14 patches from which we collected soil (table A1). Relative elevation, an approximation of the distance to the water table, was calculated as the difference in patch elevation and the elevation of the upper boundary of the nearest mapped wetland (Quintana-Ascencio et al. 2018). Time since fire, patch area, and patch aggregation were obtained from Archbold records and previous studies (see the appendix, available online; Quintana-Ascencio and Menges 1996; Menges et al. 2017).

Data Analysis. All analyses were conducted using R version 3.3 (R Development Core Team 2017). We analyzed germination and height data using generalized linear mixed effects models (binomial error) and linear mixed effects models (natural log transformation), respectively, using the lme4 package (Bates et al. 2015) after ensuring the data met the assumptions of the respective models. Both models included the microbe treatment, relative elevation, time since fire, and two-way interactions between the microbe treatment and the covariates. The rosemary scrub patch from which soil was collected was included as a random effect. For the height analysis, we also included the number of surviving plants in each pot as a covariate. For both germination and height analyses, we evaluated all possible models and selected the most likely model based on a corrected Akaike information criterion (tables A2–A4). We used a principal component

analysis of the correlation matrix to reduce the dimensionality of the soil nutrient data and overlaid vectors for relative elevation and time since fire using the envfit() function in the R vegan package (Oksanen et al. 2016).

IPM

To model the effects of soil microbes on population growth rate, we incorporated the bioassay results into an IPM. The model was initially constructed by Quintana-Ascencio et al. (2018) to project population growth rates of *H. cumulicola* in rosemary scrub patches based on a patch's unique set of environmental factors (see the appendix) but did not consider the role of soil microbes in population dynamics. The IPM was based on 22 years of annual census data from 15 populations that included 38,313 observations of 10,910 *H. cumulicola* individuals. The life cycle of the plant was divided into three stages—seed bank, yearling, and adult. Vital rate functions were constructed while accounting for four patch-level environmental covariates that varied across the landscape: elevation relative to the water table, time since fire, patch aggregation, and patch area. These vital rate functions were combined into a kernel that estimated transition rates between both size (height) and life-history stages.

We incorporated our germination data into the fecundity subkernel described in Quintana-Ascencio et al. (2018) in two ways. First, the original IPM estimated that seeds enter the seed bank at a constant rate (1 − germination rate). We replaced this constant germination rate with predictions from our most likely germination model (table A2) evaluated for a given microbial treatment and set of environmental factors associated with a rosemary scrub patch. Second, we used the same method to better estimate germination rates between the seed bank and yearling stages. We did not alter the distribution of yearling size in the original IPM, since we found no evidence of microbial effects on plant height in our bioassay (see "Results").

We used the IPM to simulate population growth rates for populations on both live and sterilized soils across the environmental conditions found in 92 rosemary scrub patches at Archbold Biological Station. Each simulation was run for the first 10 years postfire, during which fires rarely occur (Menges et al. 2017) and *H. cumulicola* population growth rates typically exhibit a hump-shaped pattern, increasing rapidly and then slowly declining (Quintana-Ascencio et al. 2003, 2018). For each postfire year in each patch, we recalculated vital rates to construct the kernel and calculated the population growth rate (λ) as the dominant eigenvalue (Ellner and Rees 2006).

To evaluate contributions of microbes and environmental factors to the variation in λ across these 92 patches, we used analysis of variance to partition its components following Sheth and Angert (2018). This approach allowed us to investigate how the predictor variables (both binary and continuous) explained the variance in λ across a realistic range of environmental conditions. Because the hump-shaped relationship between λ and time since fire was consistent across patches and differed only in height (see "Results"), we used the maximum λ value for a patch as the response variable. For each population, we modeled the (log-transformed) maximum λ value predicted by the IPM as a function of microbe presence, elevation above the water table, patch area, patch aggregation, the two-way interactions between microbes and both relative elevation and aggregation, and all interactions between the three covariates. Through comparison of the sums of squares of predictor variables, we partitioned their effects on λ across the landscape. All data from the bioassay have been deposited in the Dryad Digital Repository (https://doi.org10.5061/dryad.d7p497r; David et al. 2019), and scripts are included in a zip file, available online.[1]

Results

Bioassay

The bioassay revealed positive microbial effects on *Hypericum cumulicola* germination but no effects on plant ~~growth~~. The most likely model for germination included relative elevation to the water table, the microbial treatment, and their interaction (table A2). The presence of soil microbes nearly doubled *H. cumulicola* germination (31% ± 5% [SE] in live soils vs. 16% ± 3% [SE] in sterilized soils; $P < .001$; fig. 1*A*; table A3). Interestingly, while overall germination decreased with relative elevation to the water table (Elev: $P = .019$), the strength of the positive microbial effect on germination increased with relative elevation (Microbe × Elev: $P < .001$; fig. 1*B*). Soil analyses showed that both relative elevation and time since fire were negatively associated with soil C and N (fig. A1; figs. A1–A4 are available online). Together, these findings suggest that the benefits of microbes to germination increase as the soil becomes more nutrient poor. In contrast, we found that the most likely model for plant height included neither the microbial treatment nor any of the environmental covariates but instead only plant density, which was negatively associated with height (table A4; fig. A2).

Population Modeling

Simulations indicated that the presence of soil microbes increased population growth rate (λ) by 13%, a benefit that for most habitat patches (76%) was the difference between

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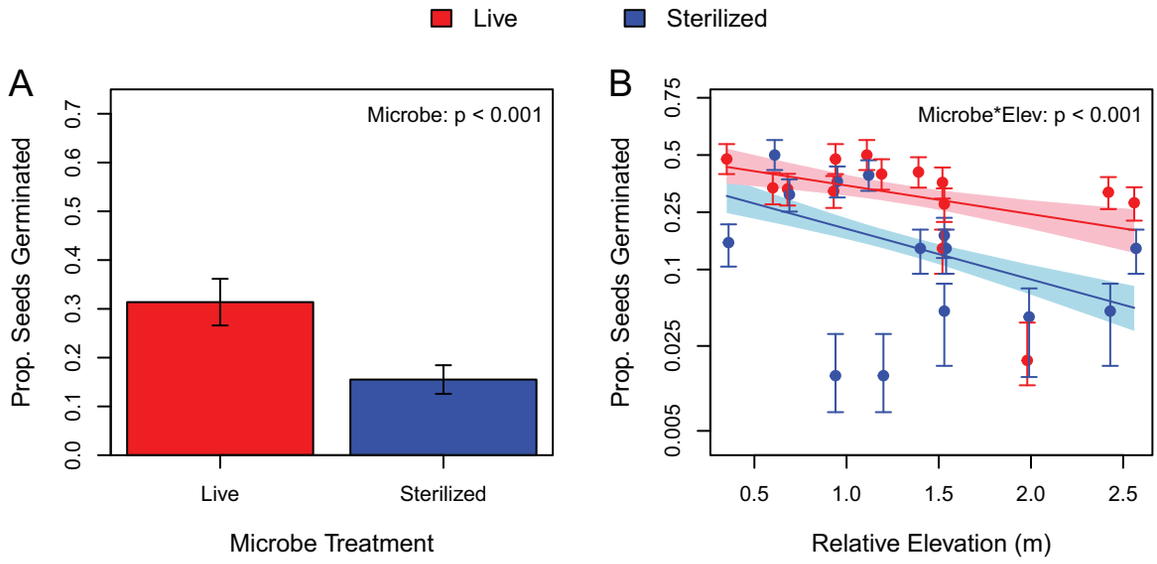

Figure 1: Bioassay results for proportion of germinating seeds. *A*, Live soil (red) increases germination compared to sterilized soil (blue; back-transformed means ± SE). *B*, Benefit of live soil to germination increases with relative elevation above the water table. Mean responses (proportion germinated) for each patch's soil ±1 binomial SE as a function of patch elevation (above the nearest wetland) plotted on a logit scale. Regression lines show microbe treatment responses across patches with standard error (shading).

a decreasing population in sterilized soil (mean maximum $\lambda = 0.95 \pm 0.07$ [SD]) and an increasing population in live soil (mean maximum $\lambda = 1.07 \pm 0.03$ [SD]; fig. 2*A*). Populations grown in the presence of soil microbes exhibited boom-bust dynamics following fire (fig. 2*A*) similar to those described by Quintana-Ascencio et al. (2018), but those populations without soil microbes rarely experienced any population growth. Soil microbes increased the number of postfire years with positive population growth, with 96% of populations in live soil experiencing ≥1 year of growth compared to 20% in sterilized soil (fig. 2*B*). This contrast was even more pronounced when considering the percentages of populations with ≥4 years of growth (84% for live vs. 1% for sterilized), indicating that without soil microbes, most populations of this endangered species would decline. Furthermore, the presence of soil microbes explained 47% of the variation in patches' maximum λ values, substantially more than relative elevation (31%), aggregation (7%), or patch area (0.1%; fig. A3), suggesting that the presence of soil microbiomes is more important than these other environmental variables for population persistence in this system.

Microbial effects were particularly influential in high-relative-elevation, low-nutrient patches where λ values were generally low (fig. 2*C*). At low relative elevation, populations in live soil had 4% higher λ than in sterilized soil, while at high relative elevation this benefit increased to 47% (fig. 2*D*). This finding suggests that *H. cumulicola* is able to occupy stressful, high-elevation patches in large part because of the beneficial effects of microbes found in these habitats.

Discussion

By scaling plant-microbe effects from individuals to populations, we have demonstrated that the presence of soil microbes can underlie plant population growth and persistence. Moreover, our results show that microbial mitigation of harsh environmental conditions for imperiled plants can be critical for allowing persistence in stressful habitats. Below we discuss how the soil microbiome shapes plant species' demography and distributions and how our findings support the integration of soil microbiomes into conservation.

Recent advances in host-microbiome research overwhelmingly show that microbes can benefit their hosts, and our study demonstrates that such individual-level effects can scale up to exert strong population-level effects. Similar work examining the consequences of microbes on plant demography is quite limited (e.g., Yule et al. 2013; Chung et al. 2015) and for belowground microbes is completely unknown (Ehrlén et al. 2016). Our findings on the benefits of the microbiome for host plant persistence agree with previous work showing that an aboveground, tightly coevolved endosymbiotic fungus is required for persistence of the rare grass *Poa alsodes* (Chung et al. 2015). Evolutionary theory predicts stronger selection for microbially conferred benefits in the fungal endosymbiont system,

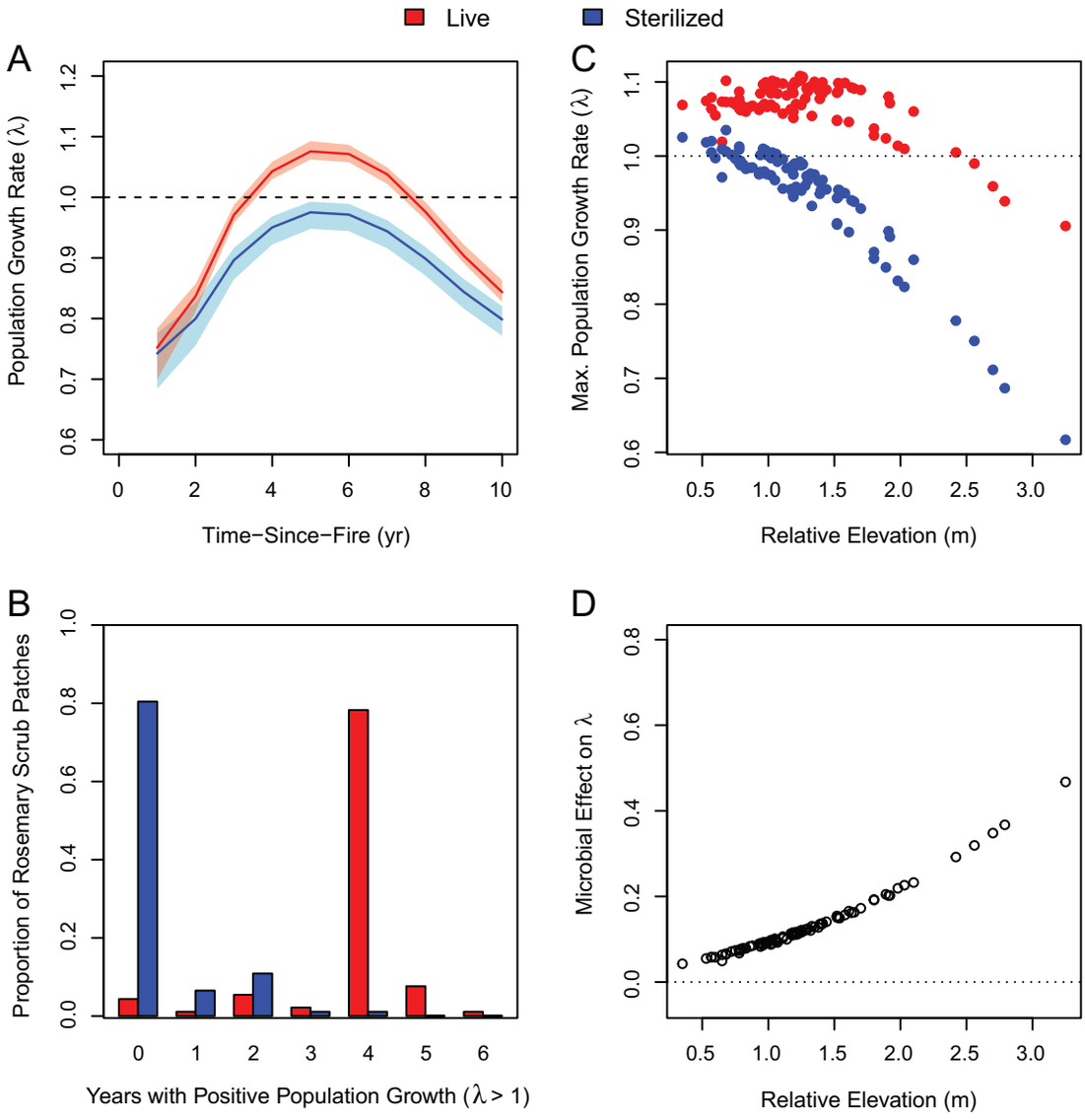

Figure 2: Population growth rates (λ) for live (red) and sterilized (blue) populations simulated using integral projection modeling. *A*, λ values for *Hypericum cumulicola* populations at 92 rosemary scrub patches projected 1–10 years postfire. Solid lines show mean λ for populations grown in live or sterilized conditions, and shading shows the 25%–75% quantile range. Dotted line shows $\lambda = 1$ (stable population growth). *B*, Histogram showing the frequency of patches predicted to attain positive population growth ($\lambda > 1$) for a given number of postfire years. *C*, Maximum λ for each patch in live or sterilized soils differs with relative elevation (above the nearest wetland). Dotted line shows $\lambda = 1$. *D*, Microbial effect on $\lambda[(\lambda_{\text{live}} - \lambda_{\text{sterilized}})/\lambda_{\text{sterilized}}]$ for each patch in *C* increases with relative elevation. The dotted line shows 0% change.

where the systemic symbiont is vertically transmitted and host and microbe fitness are thus tightly linked, than in our system where the soil microbiome is horizontally transmitted (Ewald 1987). Indeed, our mean estimate of the soil microbial effect on λ (13%) is lower than analogous estimates of the effect of systemic, aboveground endophytes on λ of their host grasses (18%–32%; Yule et al. 2013; Chung et al. 2015). However, in high-relative-elevation patches, microbial effects on λ (47%) exceed these previously published estimates, emphasizing the critical role played by soil microbiomes in certain habitats despite their transmission mode. Furthermore, the strength of microbial effects on λ can exceed those of more traditionally recognized environmental gradients (e.g., patch relative elevation, area, and aggregation; Ehrlén et al. 2016; Gurevitch et al. 2016; Quintana-Ascencio et al. 2018). While we caution that our findings are based on comparisons with sterilized soils that would not be found in a natural environment and therefore could overestimate these effects, our study

nevertheless quantifies the crucial, overlooked role that soil microbes play in plant population dynamics.

Microbes exerted strong effects on population dynamics by influencing one of the host's critical vital rates: germination. We speculate that microbes can mitigate stressful soil conditions by increasing soil moisture, thereby stimulating seed germination (e.g., Baskin and Baskin 1998). Such effects may be particularly important for germination in high-elevation patches, where soils are especially xeric (Weekley et al. 2007). While one limitation is that we could not evaluate microbial effects on later life stages of the plant, previous work (Picó et al. 2003) demonstrated that these other vital rates are less critical to population growth of *Hypericum cumulicola* (as shown through elasticity analyses). Therefore, microbial effects on these less important vital rates would have to be quite strong to similarly affect population dynamics. Importantly, many studies of soil microbiome effects on plant fitness focus on adult biomass (e.g., Kardol et al. 2006; Schnitzer et al. 2011); however, for species where adult size is not actually associated with a critical vital rate (e.g., adult survival or seed production), conclusions drawn about population-level effects could be misleading.

Species distributions can be shaped by biotic interactions that expand or contract species' ecological niches (Bruno et al. 2003). Microbes can ~~facilitate~~ or exacerbate stressors found in particular habitats, thereby expanding or reducing the habitat utilized by a given plant species and ultimately the species' distribution (Peay 2016; David et al. 2018). Our model projected population growth in stressful, higher-relative-elevation habitats only when populations were grown with microbes, suggesting that microbes expand the distribution of *H. cumulicola* into this habitat. Afkhami et al. (2014) similarly showed that by ameliorating drought stress a vertically transmitted fungus can dramatically expand a host plant range into drier regions of California; however, our present study generalizes these effects on distributions to the more common interaction between plants and soil microbiomes. Our work furthers the idea that the physiological traits that allow species to persist in a given environment (e.g., germination under stressful conditions) could in fact be microbe derived (Rodriguez et al. 2008; Peay 2016).

Finally, our finding that soil microbiomes underlie population persistence carries important implications for conservation and management. First, it suggests that a plant species cannot be conserved without also conserving its accompanying natural soil microbiome. Second, such conservation of the soil microbiome is especially important in stressful habitat and for the species endemic to that habitat. Soil amendments have long been used to reintroduce native microbes to degraded habitat (Harris 2009). Our findings suggest that such amendments could help boost plant population growth rates, particularly for species whose germination both responds positively to soil microbes and is critical to its demography. Management that relies on transplanting individuals germinated in the laboratory or greenhouse into the field could similarly benefit from germinating seeds in the presence of microbes. The soil microbiome is clearly essential for the preservation of biodiversity.

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Supplemental Methods

We briefly discuss the underlying data collection and model construction of the IPM published by Quintana-Ascencio et al. (2018) that we have built on to determine the impact of soil microbiomes on plant population dynamics.

Demographic Data

Plants from 15 Florida rosemary scrub patches were censused annually during their peak reproductive period in July–August between 1994 and 2015. Censusing resulted in 38,313 unique observations from a total of 10,910 individuals of *Hypericum cumulicola*. Plants were classified by stage (yearling vs. adult), with yearlings defined as new, untagged plants found at census time within permanent plots or plants in that patch found outside permanent plots that had a single stem that was less than 15 cm (threshold defined using an optimization algorithm that maximized correct identification and minimized mistakenly including older plants in this stage). For a subset of years, we recorded total number of reproductive structures (flowers and fruits). We used bioassays to estimate several plant reproductive metrics (fruiting, seed production, seed germination, survival to yearling stage, and seed dormancy) separately from the annual census.

Rosemary Scrub Patch Data

We calculated four environmental covariates for each rosemary scrub patch: elevation relative to the water table, time since fire, patch aggregation, and patch area. We used zonal statistics and an existing lidar data set to calculate relative elevation as the difference between the average elevation of a rosemary scrub patch and the average elevation of the wetland edges within a selected buffer surrounding that rosemary patch. Time since fire was determined using historical records of Archbold Biological Station (Menges et al. 2017). Patch area was calculated using geographic information system mapping. We calculated patch aggregation using the index provided by Hanski and Thomas (1994):

$$S_i = -\left(\sum_j^n \exp(-ad_{ij}) \times A_j\right), \tag{A1}$$

where d_{ij} is the distance in kilometers from focal patch i to patches j to n, A_j is patch area in hectares, and $a = 1$.

IPM

The basic form of the IPM consisted of a kernel ($K_{j,i}$) comprised of a survival and growth subkernel ($P_{j,i}$) and a recruitment subkernel ($F_{j,i}$; Ellner and Rees 2006):

$$K_{j,i} = P_{j,i} + F_{j,i}, \tag{A2}$$

$$P_{j,i} = \sigma_i \times \gamma_{j,i}, \tag{A3}$$

$$F_{j,i} = \text{Seed}_{j,i} + \text{Yearling}_{j,i}, \tag{A4}$$

where j is the index for the vector of size classes n at $t + 1$ and i the index at time t. The $P_{j,i}$ subkernel consisted of the survival probability (σ_i) at size i and the probability of growth ($\gamma_{j,i}$) from size i to size j. The $F_{j,i}$ subkernel predicted the number of seeds and the number of yearlings of size j produced by an individual of size i based on a composite of sequential events including seed production, dormancy, germination, and survival until reaching the yearling stage. The transition matrix consisted of a Goodman (1969) matrix model (with 301 × 301 cells; fig. 2b in Quintana-Ascencio et al. 2018) that describes the population dynamics of three stages, one discrete (dormant seeds) and two continuous

(yearlings and adults). Every transition matrix consisted of two merged rectangular matrices (of 300 × 150 cells each for yearlings and adults, respectively), one column vector for germination of dormant seeds and their survival to census as yearlings, one row vector for contributions to the seed bank, and one scalar describing long-term seed dormancy. Yearlings, by definition, were first-year plants. However, their vital rates varied based on their size, just as with adult plants, and therefore they occupy an equal percentage of the transition matrix. The IPM was integrated over $L = -2.3$ to $U = 4.5$ (logarithm values of 0.1–90 cm in height, respectively).

Recruitment of new seeds into the seed bank was modeled using estimates of seed survival in the seed bank at time $t(s(t))$, probability of reproduction (φ_0), number of reproductive structures (φ_1), fruit set (φ_2), seeds per fruit (φ_3), and the proportion of seeds going dormant (δ_E). Seeds that germinated immediately were not considered part of the seed bank:

$$\text{Seed}(y, t+1) = s(t) + \int_L^U [\varphi_0(x)\varphi_1(x)\varphi_2\varphi_3 s(t)\delta_E]dx. \quad \text{(A5)}$$

Yearling recruitment occurred either via newly produced seeds or from the seed bank. Recruitment from the seed bank was modeled using the fraction of germinating seeds ($1 - \delta_E$) that survived until monitoring based on the same factors as above and survival of germinants to the yearling stage (φ_4):

$$\text{Yearling}(y, t+1) = \text{Seed}(x, t)(1-\delta_E)\varphi_4 + \int_L^U [\varphi_0(x)\varphi_1(x)\varphi_2\varphi_3(1-\delta_E)\varphi_4]dx. \quad \text{(A6)}$$

Values of all variables were calculated for each patch using fitted mixed effects regression models based on the four covariates, with the exceptions of scalar values for fruit set ($\varphi_2 = 0.55$) and seeds per fruit ($\varphi_3 = 12.9$; see the supplemental zip file; Quintana-Ascencio et al. 2018). Prior to conducting these analyses, we checked that none of the four covariates were significantly correlated with one another (all correlations were ≤0.40). We note that φ_4 was the output of a nonlinear regression function, and this function accounts for the humped shape of the curve in figure 2*A* and explains much of the temporal variation in population growth rates. Many of the vital rates are functions of time since fire, and thus the kernel has a different value for each year postfire.

For the present study, we altered the δ_E term from the constant term used in Quintana-Ascencio et al. (2018). The probability of a seed going dormant in patch E was modeled using the coefficients (β) from the most likely model from the germination bioassay data (table A2) and was evaluated for a given patch using the patch's relative elevation (Elev) and a given microbial treatment (Microbe: 0 or 1).

$$\delta_E = 1 - \text{inverse logit}[\beta_0 + \beta_1 \times \text{Elev}_E + \beta_2 \times \text{Microbe} + \beta_3 \times \text{Microbe} \times \text{Elev}_E]. \quad \text{(A7)}$$

Although *H. cumulicola* populations immediately following fire are not in their stable size distribution, λ is still the best metric of overall population growth. To illustrate this point, we simulated a population postfire that began with 100 seeds, zero yearlings, and zero adults. For each of these three stages, we calculated the ratio of increase as N_{t+1}/N_t. As figure A4 shows, the dynamics of any individual stage are fairly complex, but λ represents an average of the three stages. In particular, the dynamics of the adult stage converge with λ fairly quickly, such that they are almost perfectly matched by the fourth year.

Table A1: Description of patches from which soils were collected for the bioassay

Patch ID	Relative elevation (m)	Time since fire (years)	Area (h)	Aggregation	Total C (%)	Total N (%)	Total P (mg/kg)	Total K (mg/kg)	Long-term monitoring
1	1.98	17	.56	2.85	.41	.01	2.1	7.67	Yes
4	.93	0	.17	2.59	.55	.02	1.36	11.86	No
13	1.52	15	.08	3.28	.43	.01	1.66	5.74	No
29	.6	1	1.40	3.68	.34	.01	1.68	6.1	Yes
32	.68	19	.22	4.83	.22	.01	1.79	5.08	Yes
37	2.56	19	.13	5.56	.29	.02	1.73	4.53	No
39	2.42	19	.14	5.9	.18	.00	1.98	4.51	No
42	1.19	6	1.70	7.66	.52	.01	1.54	3.8	Yes
45	.35	15	.23	5.37	1.09	.04	1.78	5.73	Yes
88	1.11	8	.27	10.3	.37	.02	1.76	5.33	Yes
92	1.39	30	.35	7.23	.46	.00	2.28	10.44	No
94	.94	6	.06	10.1	.77	.02	1.4	7.19	No
6054	1.52	6	3.59	8.87	.87	.03	1.79	6.39	No
9591	1.53	30	.35	9.55	.22	.00	1.48	4.59	No

Note: Patch ID refers to the official Archbold Biological Station identification. Relative elevation and time since fire data were accessed using Archbold's grid database (Menges et al. 2017). Patch aggregation, a function of distance to other patches and their area, was previously calculated by Quintana-Ascencio and Menges (1996). Total C, N, P, and K of soils were analyzed in the present study (fig. A1). Patches were chosen to represent a range of relative elevation (across 92 patches: mean ± SD = 1.26 ± 0.51 m, min = 0.35 m, max = 3.25 m), patch area (mean ± SD = 0.436 ± 0.550 ha, min = 0.003 ha, max = 3.594 ha), and patch aggregation (mean ± SD = 7.45 ± 3.48, min = 2.16, max = 11.8; Quintana-Ascencio et al. 2018). For time since fire, we selected patches that provided a range of fire return intervals relevant to *Hypericum cumulicola*, which tends to boom and bust during the first 10 years following fire. Menges et al. (2017) calculated the percentages of all patches at Archbold Biological Station in a given fire return interval: 2–10 years (15%), 10–19 years (35%), 20–59 years (45%), and 60–100 years (5%). Several of the patches selected contained long-term monitoring populations of *H. cumulicola* (Quintana-Ascencio et al. 2018).

Table A2: Model selection for *Hypericum cumulicola* germination

Term	df	AICc	Δ
Elev + Microbe + Microbe × Elev	**4**	**2,158.59**	**.00**
Elev + Microbe + TSF + Microbe × Elev	5	2,159.82	1.24
Elev + Microbe + TSF + Microbe × Elev + Microbe × TSF	6	2,160.54	1.95
Elev + Microbe + TSF + Microbe × TSF	5	2,168.75	10.17
Elev + Microbe	3	2,172.03	13.45
Elev + Microbe + TSF	4	2,173.21	14.63
Microbe + TSF + Microbe × TSF	4	2,308.98	150.39
Microbe + TSF	3	2,313.27	154.68
Microbe	2	2,328.48	169.90
Elev	2	2,368.74	210.16
Elev + TSF	3	2,369.94	211.35
TSF	2	2,505.41	346.82
Null	1	2,520.08	361.50

Note: The global model was fitted using a generalized linear mixed effects model with binomial error. The rosemary scrub patch from which soil was collected (soils from 14 patches were used in the bioassay) was used as a random effect. We included the microbe treatment (Microbe; sterilized vs. live), relative elevation above the nearest wetland (Elev), time since fire (TSF), and the two-way interactions between Microbe and the covariates. All covariates were standardized (mean = 0, SD = 1) prior to analysis. The boldface row depicts the most likely model using a corrected Akaike information criterion (AICc). Δ is the difference in AICc between each model and the model with the lowest AICc.

Table A3: Model coefficients for analysis of *Hypericum cumulicola* germination

Term	Estimate	SE	*P*
Intercept (β_0)	−.76	.19	...
Elev (β_1)	−.29	.16	.019
Microbe (β_2)	−.96	.07	<.001
Microbe × Elev (β_3)	−.22	.06	<.001

Note: See table A2 for a description of model terms. *P* values were obtained using analysis of deviance.

Table A4: Model selection for analysis of *Hypericum cumulicola* height

Term	df	AICc	Δ
Plants	**4**	**91.90**	**.00**
Plants + TSF	5	92.00	.10
Plants + Soil	5	93.95	2.05
Plants + Soil + TSF	6	94.11	2.21
Plants + Elev	5	94.11	2.21
Plants + Elev + TSF	6	94.26	2.36
Plants + Elev + Soil + Soil × Elev	7	95.15	3.25
Plants + Elev + Soil + TSF + Soil × Elev	8	95.42	3.52
Plants + Elev + Soil	6	96.26	4.36
Plants + Elev + Soil + TSF	7	96.39	4.49
Plants + Soil + TSF + Soil × TSF	7	96.52	4.62
Plants + Elev + Soil + TSF + Soil × Elev + Soil × TSF	9	97.04	5.14
Plants + Elev + Soil + TSF + Soil × TSF	8	98.87	6.97
Null	3	139.56	47.66
Elev + Soil + Soil × Elev	6	141.17	49.27
TSF	4	141.30	49.40
Elev	4	141.40	49.50
Soil	4	141.42	49.52
Elev + TSF	5	142.55	50.65
Elev + Soil + TSF + Soil × Elev	7	142.55	50.65
Soil + TSF	5	143.21	51.31
Elev + Soil	5	143.40	51.50
Elev + Soil + TSF	6	144.66	52.76
Elev + Soil + TSF + Soil × Elev + Soil × TSF	8	144.86	52.96
Soil + TSF + Soil × TSF	6	145.31	53.41
Elev + Soil + TSF + Soil × TSF	7	146.83	54.93

Note: The global model was fitted using a linear mixed effects model. Mean stem height of surviving plants in each pot was natural log transformed prior to analysis. The rosemary scrub patch from which soil was collected (soils from 14 patches were used in the experiment) was used as a random effect. We included the microbe treatment (Microbe; sterilized vs. live), relative elevation above the nearest wetland (Elev), time since fire (TSF), the two-way interactions between Microbe and the covariates, and the number of plants surviving in the pot (Plants). All covariates were standardized (mean = 0, SD = 1) prior to analysis. The boldface row depicts the most likely model using a corrected Akaike information criterion (AICc). Δ is the difference in AICc between each model and the model with the lowest AICc.

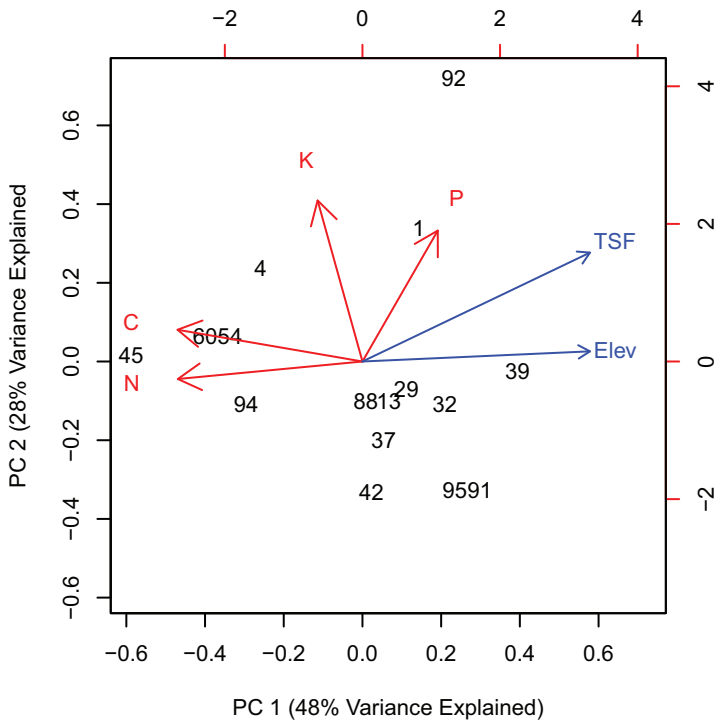

Figure A1: Principal component analysis for soil properties (total C, N, P, K) of soils collected from 14 rosemary scrub patches at Archbold Biological Station. All metrics were mean centered around zero with a standard deviation of one to account for different units. Numbers represent patch IDs, and red vectors depict each element. Vectors for elevation relative to the water table (Elev) and time since fire (TSF) were overlaid. C and N concentrations were negatively associated with TSF and Elev.

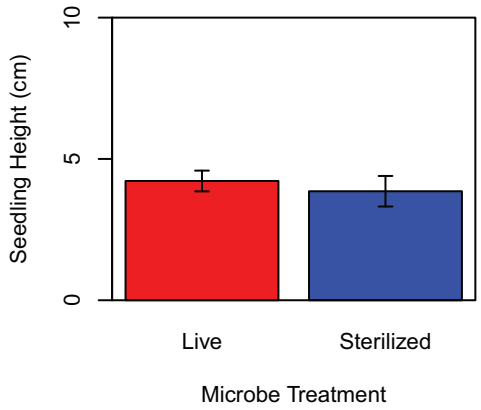

Figure A2: Bioassay result for plant height (back-transformed means ± SE) of *Hypericum cumulicola* in the live and sterilized microbe treatments. The microbe treatment was not included in the most likely model (table A3).

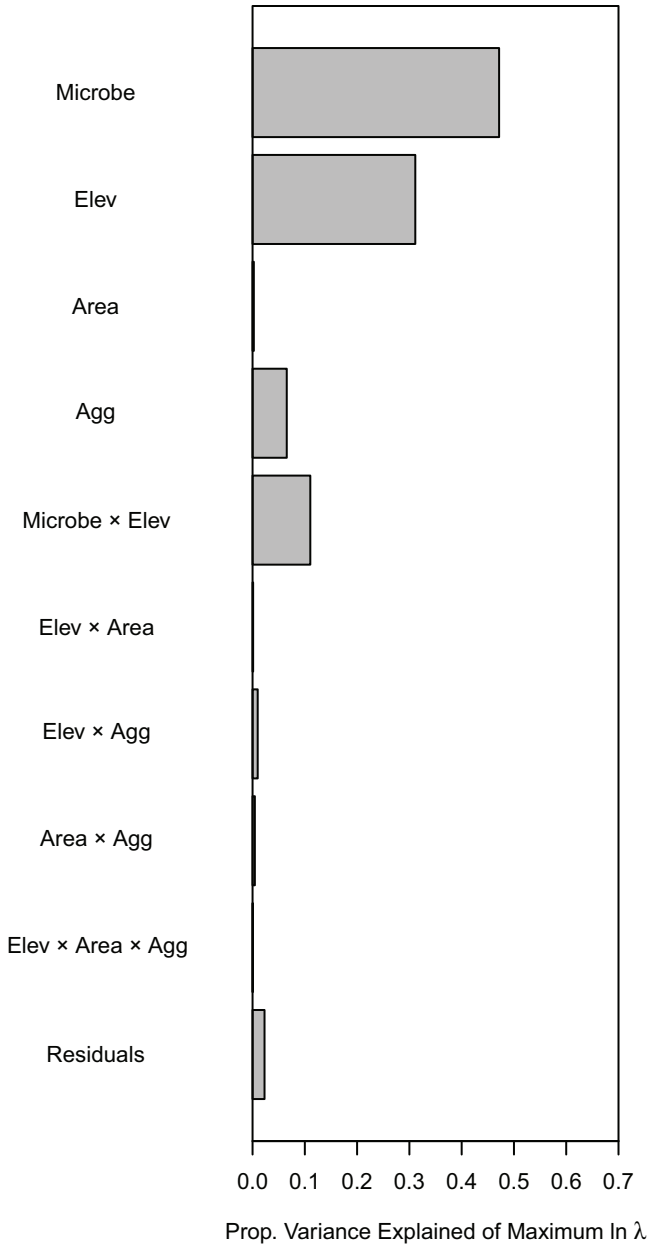

Figure A3: Variance components analysis of environmental factors that predict variation in maximum *Hypericum cumulicola* λ values across 92 rosemary scrub patches at Archbold Biological Station. The maximum λ value of each patch typically occurred in years 5 or 6 postfire.

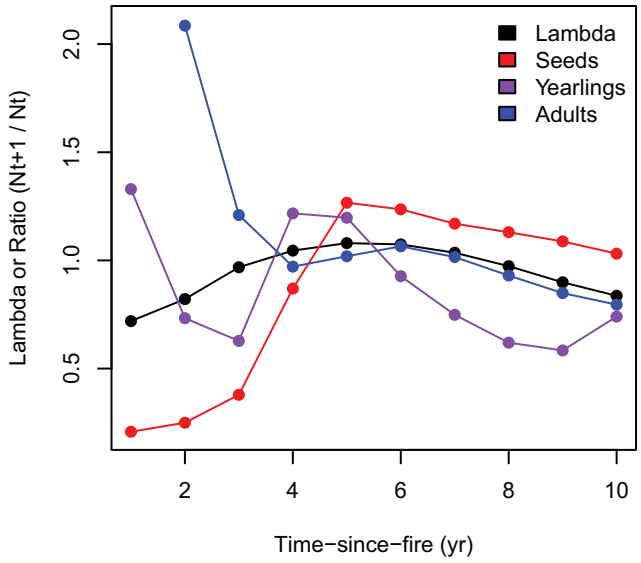

Figure A4: Comparison between asymptotic population growth rate (λ) and the ratio of increase for each of the three life stages (seed bank, yearling, adult). The simulation was parameterized by setting elevation, area, and aggregation to zero (mean of each mean-centered covariate) and used initial starting values of 100 seeds, zero yearlings, and zero adults. The plot shows that λ acts as an average of the dynamics experienced by the different life stages. In particular, there is a boom of adults immediately postfire, but this is at the expense of depleting the seed bank until adults have grown large enough to replenish it.

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