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Costs, benefits, and loss of vertically transmitted
symbionts affect host population dynamics. – Oikos
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Appendix 1

Abiotic and biotic characteristics of the sites of experimental and natural *Agrostis hyemalis* populations

Natural populations

Two natural populations of *Agrostis hyemalis* at Stephen F. Austin Experimental Forest were chosen in order to compare environmental variables with those of the experimental site. These populations were on rocky soils located in early-successional, disturbed areas near roadsides. Natural population A was located in an understory habitat ~ 0.16 km from the experimental plots, and was the source population for the seeds used in the experiment (31°29'58"N, 94°46'01"W). Natural population B was farther (1.37 km) from the experimental site in an open and sunny area and contained a high density of flowering *A. hyemalis* plants (31°30'29"N, 94°46'23"W).

Environmental variables

We collected information on environmental variables on 23 April 2012. We measured the photosynthetically active radiation (PAR) with an linear ceptometer and volumetric water content

(VWC) with a soil moisture probe at twenty randomly placed locations in each of the two natural population sites and at two randomly placed locations in each of the experimental plots. Additionally, we measured plant species richness and visually estimated the percentage cover by vegetation (PCV) within two randomly placed 0.5×0.5 m quadrats within each experimental plot and six randomly placed quadrats within each of the sites of natural populations. We performed one-way ANOVAs in SAS to test for effects of site on these environmental variables (Table A1). Due to the denser canopy cover, the experimental plots were shadier and had lower plant species richness within the sampled quadrats than the sites of the natural populations, supporting the idea that they represented a later successional stage. However, levels of moisture and total percent cover by understory vegetation did not differ significantly between the experimental site and the site of natural population A.

Soil analyses

All soil samples were collected 23 April 2012. Three soil samples, each of which consisted of an aggregation of soil from under five *A. hyemalis* individuals, were taken from each of the two natural population sites, and one soil sample was taken from a haphazardly chosen location within each of the experimental plots. Analyses of soil pH, conductivity, and macro and micro nutrients for each sample were completed by the Texas A&M University (<http://soiltesting.tamu.edu/>). We performed one-way ANOVAs in SAS to test for effects of site on soil characteristics (Table A1). The levels at the experimental site did not differ statistically from those at natural site B for any soil characteristic, excepting S. However, the concentrations of several nutrients at the experimental site were significantly different from those at natural site A, despite the close geographic proximity of the sites.

Table A1. Means \pm SE and results from one way ANOVAs of site environmental variables and soil nutrient levels for the sites associated with three *Agrostis hyemalis* populations. Significant differences in mean values between sites by post hoc Tukey HSD test are indicated by differing superscript letters ($\alpha = 0.05$).

	Site means \pm SE			DF	F	p
	Experimental	Natural A	Natural B			
PAR ¹ ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	178 \pm 42 ^a	738 \pm 123 ^b	898 \pm 105 ^b	2,57	22.91	<0.0001
VWC (%)	9.8 \pm 0.85 ^a	13.6 \pm 0.85 ^a	5.1 \pm 0.85 ^b	2,57	24.90	<0.0001
PCV ¹ (%)	17 \pm 5 ^a	37 \pm 8 ^{a,b}	56 \pm 8 ^b	2,29	8.54	<0.0001
Plant species richness	2.1 \pm 0.2 ^a	4.3 \pm 0.4 ^b	5.5 \pm 0.4 ^b	2,29	32.2	<0.0001
pH ¹	4.5 \pm 0.3 ^a	5.0 \pm 0.5 ^a	4.9 \pm 0.5 ^a	2,15	0.90	0.4301
EC ^{1,2} ($\mu\text{mhos cm}^{-1}$)	90 \pm 17 ^a	100 \pm 31 ^a	44 \pm 31 ^a	2,15	3.10	0.0794
NO ₃ ⁻ -N (ppm)	0.58 \pm 0.17 ^a	0.45 \pm 0.31 ^a	1.29 \pm 0.31 ^a	2,15	2.29	0.1404
P (ppm)	13.6 \pm 0.8 ^a	6.8 \pm 1.5 ^b	11.4 \pm 1.5 ^{a,b}	2,15	8.21	0.0050
K (ppm)	59 \pm 5.9 ^a	103 \pm 10.7 ^b	78 \pm 11 ^{a,b}	2,15	7.05	0.0085
Ca ¹ (ppm)	776 \pm 363 ^a	998 \pm 662 ^a	437 \pm 662 ^a	2,15	1.00	0.3936
Mg (ppm)	101 \pm 15.9 ^a	245 \pm 30.0 ^b	86.7 \pm 30.0 ^a	2,15	10.72	0.0018
S (ppm)	10.3 \pm 0.823 ^a	6.65 \pm 1.50 ^{a,b}	4.62 \pm 1.50 ^b	2,15	6.58	0.0106
Na (ppm)	16.8 \pm 0.566 ^a	12.7 \pm 1.03 ^b	18.4 \pm 1.03 ^a	2,15	8.59	0.0042
Fe (ppm)	136 \pm 30.7 ^a	86.7 \pm 56.1 ^a	25.7 \pm 56.1 ^a	2,15	1.57	0.2456
Zn ¹ (ppm)	2.7 \pm 0.54 ^a	1.6 \pm 1.0 ^a	2.1 \pm 1.0 ^a	2,15	0.30	0.7488
Mn (ppm)	46 \pm 6.9 ^a	21 \pm 13 ^a	21 \pm 13 ^a	2,15	2.47	0.1230
Cu ¹ (ppm)	0.27 \pm 0.05 ^a	0.23 \pm 0.09 ^a	0.15 \pm 0.09 ^a	2,15	1.47	0.2662

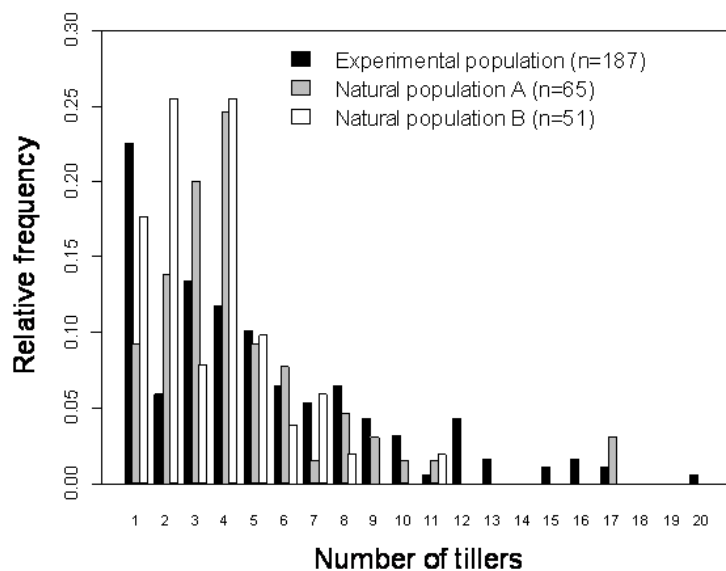
¹log-transformed for the analysis to meet ANOVA assumptions of normality and homogeneity of variance, means given are not transformed

²electrical conductivity of the soil

Plant size distributions

On 23 April 2012, during the peak flowering time and before seed production, we measured the number of tillers on all of the *A. hyemalis* plants in natural population A (n = 65) and a subset in natural population B (n = 51), which had a high density of *A. hyemalis* plants. The distributions of the log-transformed tiller counts from these two populations were then compared with the 13 and 14 May 2009 log-transformed tiller count from the experimental population (n = 187) using a Kolgorov-Smirnov test. The size distributions of natural population A and the experimental population did not differ significantly ($D = 0.1456$, $p = 0.258$), while the natural population B exhibited less variation in size ($D = 0.2299$, $p = 0.0289$) (Fig. A1). Therefore, we conclude that, although begun from adult plants grown in a greenhouse, our experiment was not biased by an unrealistic size distribution.

Figure A1. Size distributions of *Agrostis hyemalis* plants in the experimental population, natural population A, and natural population B.



Appendix 2

Further details on response variables for demographic data on *Agrostis hyemalis*

Survival, size and growth

Plant survival and size (estimated by counting the total number of tillers) was recorded for every plant in 2008 (1 May), 2009 (13 and 14 May), and 2010 (1 March and 29 April).

Reproduction

The total number of seeds per plant was calculated as (number of inflorescences) \times (estimated number of seeds per inflorescence). In 2008, plants produced many flowering tillers over a long time interval. Thus, the number of inflorescences per plant was recorded on 5 Mar 2008 and again on 1 May 2008; the larger count between the two dates was used for each plant as an estimate of the number of inflorescences. On 29 April and again on 20 May 2008, one inflorescence was haphazardly collected per plant from which the number of seeds and the number of empty spikelets (indicating dispersed seeds) were counted (in *A. hyemalis* each spikelet produces one flower). The sum of the seeds and empty spikelets was averaged for the two inflorescences per plant, resulting in an estimate of the number of seeds per inflorescence. In 2009, fewer inflorescences were produced and the flowering period was shorter (13–25 May 2009). Therefore, the estimated number of seeds per inflorescence was obtained from only one haphazardly chosen inflorescence per plant. In 2010, this process was repeated using maximum counts of seeds per inflorescence and the number of inflorescences per plant from 31 Mar 2010 and 29 Apr 2010. The total number of seeds per plot for each year was estimated by summing the estimated seeds for all plants in the plot and subtracting any seeds removed to estimate germination and vertical transmission). Seeds that were removed for estimating seed production were redistributed within the plot from which they were removed to allow for natural recruitment.

Seedling establishment and size

Seedlings that recruited within a plot were counted and tagged. On 15 May 2009, the first recruit cohort was censused, the tillers were counted, and the number of seeds produced was estimated for each recruit. On 29 Apr 2010, the survival, total number of tillers, and number of flowering tillers was recorded for the 2009 recruits, and 2010 recruits were counted and tagged. Because recruits were small (1–2 leaves), scoring endophyte status would have been destructive, preventing us from following recruits into the next year. Thus, we took the conservative approach of assuming all new recruits had the same endophyte status as the plot in which they recruited, maximizing our ability to track the survival and growth of recruits.

Vertical transmission rate

We removed a subset of seeds from collected inflorescences, germinated these on agar plates, and checked seedlings for endophytes by staining thin sections of inner leaf sheath with aniline blue-lactic acid stain (Bacon and White 1994) and examining the tissue with a compound brightfield microscope at 200–400 \times . Vertical transmission rates were calculated as (number of E+ seedlings) / (total number of seedlings scored). In 2008, ten seedlings were checked from each E+ plot ($\tau = 0.78 \pm 0.07$). Due to the variation in plot-level transmission rates found in 2008, we decided to check a greater number of seedlings in 2009 in order to gain a better estimate of vertical transmission rates. Due to variation in plot-level seed production and germination in the greenhouse, a mean of 48.2 seedlings were checked from each E+ plot [range 7–64] in 2009 ($\tau = 0.31 \pm 0.05$). We were motivated to model the potential effects on the system of the entire range of possible vertical transmission rates by the variability between plots and across years in the estimates of transmission and the low confidence we had in our estimates of transmission due to small sample sizes.

Reference

Bacon, C. W. and White, J. F. Jr. 1994. *Biotechnology of endophytic fungi of grasses*. – CRC Press.

Appendix 3

Life table response experiment (LTRE) of endophyte effects on *Agrostis hyemalis*

Life table response experiment (LTRE)

We used a life table response experiment (Caswell 2001) to decompose endophyte effects on λ into contributions from individual parameters (coefficients of the demographic functions). The total difference in λ reflects the difference in parameter values (Δp) multiplied by the parameter sensitivity and summed over all parameters that differed between E– and E+ populations:

$$\Delta \lambda \approx \sum_i \Delta p_i \frac{\partial \lambda}{\partial p_i} \Big|_{p_i} \quad (\text{A1})$$

Sensitivities were evaluated using the mean of E+ and E– parameters. We computed parameter sensitivities numerically by applying a uniform perturbation and taking the ratio of the change in λ to the magnitude of the perturbation (0.01). The individual parameter contributions summed to the total difference in λ , as expected.

Reference

Caswell, H. 2001. Matrix population models. – Sinauer.