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Trait plasticity, not values, best corresponds with woodland plant success in novel and manipulated habitats

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Abstract

Aims
The clustering of plants with similar leaf traits along environmental gradients may arise from adaptation as well as acclimation to heterogeneous habitat conditions. Determining the forces that shape plant leaf traits requires both linking variation in trait morphology with abiotic gradients and linking that trait variation with plant performance under varying abiotic conditions. Across the spectrum of plant types, shade-tolerant evergreen herbs are relatively low in trait plasticity, compared to deciduous and sun-adapted species. These plants employ stress-tolerant strategies for survival, which coincide with relatively static trait morphologies, slow growth and hence a lower ability to adjust to changing environmental conditions.

Methods
We investigate how the survival of two ecologically similar understory evergreen species, *Asarum arifolium* and *Hepatica nobilis*, corresponds with variation in six commonly measured functional traits (leaf area, specific leaf area, plant height, leaf number, leaf length and shoot mass) along natural and experimental abiotic gradients. We examine temporal (the period 2007–9) and spatial (100 km) variations in these traits after (i) translocating 576 plants across a span from the southern Appalachian Mountains in NC, USA, to the Piedmont, GA, USA, which includes north- and south-facing slope habitats and (ii) the experimental manipulation of diffuse light and soil moisture.

Important findings
We find that when translocated into a novel habitats, with novel environmental conditions that often are more extreme than the source habitat, both species appear capable of considerable morphological acclimation and generally converge to similar trait values. *Hepatica nobilis* does not exhibit mean trait values particularly different from those of *A. arifolium,* but it demonstrates much greater phenotypic plasticity. These results indicate that relatively conservative plant species nonetheless acclimate and survive across heterogeneous environmental conditions.

Keywords: Functional traits • *Asarum arifolium* • *Hepatica nobilis* • niche • specific leaf area • understory

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INTRODUCTION

Environmental gradients shape species distributions by how well individual niche requirements correspond with environmental heterogeneity (e.g. Ackerly and Cornwell 2007; Weiher and Keddy 1995). Plant functional traits are morphological or physiological characteristics that govern the fitness (e.g. survival) of a species by affecting growth, reproduction and survival (Reich *et al.* 2003; Violle *et al.* 2007). Sessile organisms must employ phenotypic plasticity (acclimation) to persist in the spatial and temporal heterogeneity of natural habitats (Bradshaw 1965; Lavorel and Garnier 2002; Rozendaal *et al.* 2006), so species traits may converge along environmental gradients as the plants acclimate to similar conditions. Observed similarities in species and trait clustering along environmental gradients probably reflect a combination of habitat suitability and phenotypic acclimation (Ackerly and Cornwell 2007; Ackerly *et al.* 2002; Keddy 1992; McGill *et al.* 2006; Shipley *et al.* 2006; Valladares *et al.* 2007).

Transplant studies indicate that most plants exhibit strong plasticity when exposed to varying environments (e.g. Doust 1981, 1987; Thompson *et al.* 1991a, 1991b), but plants also maintain specific trait values indicative of their individual species and ecotypes (e.g. Reich, Walters *et al.* 1998; Rozendaal *et al.* 2006; Valladares *et al.* 2000). It has been recognized that a link between an organism's niche and the environment exists in its morphological traits (Darwin 1859; Diamond 1975; Hutchinson 1959), but it may not be the presence of the exact trait values, but the flexibility of those trait values, which allows greater adaptability in novel habitats. Greater trait plasticity allows more flexibility for persistence in difficult conditions (Bongers and Popma 1988; Grassein *et al.* 2010; Griffith and Sultan 2005; Grubb 1998; Rozendaal *et al.* 2006), potentially resulting in larger realized distributions (Baeten *et al.* 2010; Donohue *et al.* 2001; Valladares *et al.* 2007). For this reason, it is important to assess plant performance in conjunction with plasticity. Comparative investigation into trait-based niche differentiation between plant species requires determining the links between environment, phenotypic plasticity, trait morphology and plant fitness (Chu *et al.* 2009; McGill *et al.* 2006).

We employ a large-scale translocation of two understory evergreen herbs, *Asarum arifolium* and *Hepatica nobilis*, across natural and manipulated abiotic gradients to investigate the correspondence between habitat-specific survival and variation in phenotypic traits. Specifically, we ask whether the species maintain specific trait values regardless of transplant environment and whether the traits, or changes in those traits, relate to survival across transplant environments. *Hepatica nobilis* fitness (as indicated by greater growth, reproduction and survival) in transplant experiments is much higher than that of *A. arifolium* (Warren 2010; Warren and Bradford 2011). Although previous work established species-specific differences in *H. nobilis* and *A. arifolium* fitness (Warren 2010; Warren and Bradford 2011), here we investigate species-specific variation in trait values and plasticity in experimental transplants. Given that an investment in tough, thick evergreen leaves generally correlates with relatively low phenotypic plasticity (Grime and Mackey 2002; Reich *et al.* 1992, 1997; Skillman *et al.* 1996; Westoby *et al.* 2002; Wright *et al.* 2004), we expect that *H. nobilis* exhibits greater survival ability because it has inherent trait characteristics better suited for the experimental habitats used in this research. Alternately, given that high phenotypic plasticity is considered integral for plant functioning in response to heterogeneous environments (Bongers and Popma 1988; Rozendaal *et al.* 2006), *H. nobilis* may perform better when transplanted into novel habitats due to greater overall trait plasticity than *A. arifolium*. If the traits chosen for this research are not linked with survival or if the degree of variation in the environmental gradients is not sufficient to induce or influence the traits, we expect little trait plasticity and no correspondence with survival.

MATERIALS AND METHODS

Study species

The transplant species are two small (10 to 15 cm tall), perennial understory evergreen herbs that occur in the temperate deciduous forests of eastern USA. *Asarum arifolium* Michx. (Aristolochiaceae; synonym *Hexastylis arifolia*) occurs in southeastern USA from Florida to Virginia, NC, to the Mississippi River (Appendix 1). *Hepatica nobilis* Schreb. var. *obtusa* (Pursh) Steyerm. (Ranunculaceae) occurs in the USA from northern Florida to Nova Scotia west, to Alabama and Missouri and Montana, as well as in Europe and Asia (Appendix 1). Both plants typically occur on north-facing slopes, depend on ants for propagule dispersal and produce no clonal offspring (Giladi 2004; Harris 2000; Warren 2007, 2008). New leaves emerge in early spring and turn over each year; however, the photoharvest efficiency of understory evergreen herbs declines substantially before senescence (Neufeld and Young 2003).

Experimental plots and treatments

In January 2006, we collected *A. arifolium* (n = 288) and *H. nobilis* (n = 288) individuals at Whitehall Forest (WHF) in Georgia, USA (33°53′N, 83°21′W) for the study. Whereas phenotypic plasticity research characteristically is focused on trait development in genetically identical individuals, its investigation has expanded to include the response of different species and populations in their ecological context (Valladares *et al.* 2006). We collected individuals from the same population to minimize genetic variability. Because key demographic functions are dependent on size rather than age for both species (Giladi 2004; Warren 2007) and because phenotypic plasticity varies as a function of ontogeny in plants (Coleman *et al.* 1994), we chose only adult, reproductive-size plants (Giladi 2004; Warren 2007). We translocated plants to experimental grids located on north- and south-facing slopes at WHF and Coweeta Hydrologic Laboratory (CWT) in NC, USA (35°03′N, 83°25′W).

Half of all transplants were subjected to watering and shading experiments. We augmented soil moisture by delivering water from two 200-l reservoirs via drip irrigation (Dripworks, Inc., Willits, CA, USA) to the lower (slope position) two rows of each grid. We delivered ~70 l of water weekly per plot during May–August 2006. To attenuate solar irradiation, we randomly assigned custom-made polyvinyl chloride frames (1 × 1 × 0.5 m) to half the plots in each grid. Black, knitted 60% neutral shade cloth (International Greenhouse Co., Georgetown, IL, USA) was attached to the top of each frame.

Ambient soil moisture, diffuse light and temperature were monitored throughout the experiments. We measured volumetric soil moisture percentage in each cell with a handheld Hydrosense Soil Water Content Measurement System (Campbell Scientific, Inc., Logan, UT, USA). We calculated incident photosynthetically active radiation (diffuse light, wavelength: 400–700 nm) as the percentage of photosynthetic photon flux density (PPFD) at a fully exposed reference site and PPFD transmitted through the forest canopy at each cell. The understory grid measurements were taken with a handheld AccuPAR ceptometer (Decagon Devices, Inc. Pullman, Washington, DC, USA), and the open reference measurements were taken simultaneously with an LI-200 spherical PAR sensor and logged with a LI-1400 datalogger (LiCor, Inc., Lincoln, NE, USA). We sampled soil moisture at five averaged points within each cell (n = 144) in June and July 2007, January 2008 and April 2009; diffuse light was sampled in each cell (n = 144) in July and January 2007 and 2008. See Warren (2010) and Warren and Bradford (2011) for more detailed description of transplant, treatment and physical monitoring methodology.

Functional traits

We utilized six aboveground plant traits commonly used in ecological investigation: leaf area (LA), specific leaf area (SLA), plant height (HGT), leaf number (LN), leaf length (LNG) and aboveground shoot biomass (SM) (Chapin *et al*. 1987; Lusk *et al*. 2008; Poorter *et al*. 2008; Shipley 2006; Weiher *et al*. 1999; Westoby *et al*. 2002; Wright *et al*. 2004). One individual plant per species per plot was selected at random and harvested in February 2007 (n = 279) and 2009 (n = 212). Photosynthesis in understory evergreen herbs is minimal at the end of their leaf span (Neufeld and Young 2003), so harvesting the leaves in February probably imposed little deleterious impact as new leaves emerge in March. Leaf area (in cm^2) was the one-sided projected leaf and petiole surface area as measured using a LI-3100C leaf area meter (Licor, Inc.). We dried the plants at 60°C for 72 h and weighed the leaves for SM (g). We calculated SLA ($\mathrm{cm}^2\ \mathrm{g}^{-1}$) by dividing LA by SM. We measured H (approximate petiole length in cm) after harvest.

Data analysis

Analysis of variance (ANOVA) models were used to assess the impacts of discrete factors on functional traits (LA, SLA, HGT, LN, LNG and SM). The factors were species identity (*A. arifolium*, *H. nobilis*), experimental watering (±), experiment shading (±), slope aspect (north, south) and site (CWT, WHF). Because a full array of interaction terms for five factors would be unfeasible and the a priori target was species-level effects, we investigated all Species × Functional trait interactions and selected the strongest statistically significant interaction for evaluation using the R statistical programming environment (R Development Core Team 2012). We determined the strongest interaction term using Akaike's Information Criterion (AIC) values (Akaike 1973). AIC measures how well the model explains variance in the data whilst penalizing the model for extraneous parameters. We reported main effects for species-level differences in all functional traits and significant main and interaction effects for treatment and landscape effects.

A relative distance plasticity index (RDPI, Valladares *et al*. 2006) was calculated for each trait across slope aspects (north and south) and years (2007 and 2009). We used the RDPI, which ranges from zero (no plasticity) to one (maximum plasticity), to examine phenotypic distances among individuals of each species (see Valladares *et al*., 2006, for full methodology). We transplanted *A. arifolium* and *H. nobilis* into north- and south-facing habitats to investigate spatial plasticity because shifts across slope aspect correspond with considerable natural contrast in environmental conditions (Warren 2010), understory evergreen herb abundance (Warren 2008) and survival between study species (Warren 2010; Warren and Bradford 2011). The RDPI generally is used to evaluate trait plasticity across experimental treatments or environmental conditions (e.g. Grassein *et al*. 2010; Valladares *et al*. 2006); we used it to evaluate plasticity across sites to compare the ability of each study species to adjust to the novel conditions in proximate (WHF) and distant (CWT) translocations. We calculated pairwise phenotypic plasticity distances as the absolute value of the difference between trait values of 2007 and 2009 in randomized individuals divided by the sum of the paired trait values (see Valladares *et al*., 2006, for full calculations). The RDPI approach can generate pairwise replicate measures of plasticity distance, which allows for robust statistical comparison between species. Inter- and intraspecific variability in plasticity values of 2007 and 2009 were compared using mean values ±90% confidence intervals (CIs). As ecological field studies pose a challenge in balancing between poor specificity (Type I error) and detection power (Type II error), we considered coefficients with P values <0.05 as significant and coefficients with P values <0.10 as marginally significant (sensu Hurlbert and Lombardi 2009).

RESULTS

Soil moisture was higher and temperature lower on north- (moisture: 18.9 ± 0.3%; temperature: 15.1 ± 0.2°C) than south-facing (moisture: 17.9 ± 0.4%; temperature: 17.9 ± 0.4°C) slopes and at CWT (moisture: 19.9 ± 0.4%; temperature: 14.1 ± 0.1°C) than at WHF (moisture: 16.9 ± 0.3%; temperature: 17.4 ± 0.1°C; see Warren, 2010, for extensive site and treatment characteristics). Summer season diffuse light (1.8 ± 0.1%) did not differ by aspect or site. Watering more than doubled the soil moisture from 13.9 ± 5.8% in unwatered plots to 29.9 ± 8.3% in watered plots and shading halved diffuse light from 2.5 ± 1.0% in unshaded plots to 1.1 ± 0.5% in shaded plots.

Hepatica nobilis consistently exhibited higher survival than *A. arifolium* across treatments, landscapes and years (Appendix 2). Both species survived much better with watering in 2007, but shading had little effect. Both species also survived much better on north- than south-facing slopes in 2007 and 2009, but only *H. nobilis* seemed significantly affected by transplant location as it survived better at CWT than WHF in 2009. The highest survival rates for both species occurred with watering in 2007 and on north-facing slopes in 2007 and 2009. The lowest survival for both species occurred on south-facing slopes in 2007 and 2009.

Across all sites and treatments, all six mean trait values differed significantly between species (Table 1, a–f) during the first year after transplanting (2007) as *A. arifolium* had lower LA, SLA, LN and SM and larger HGT and LNG than *H. nobilis* (Fig. 3a–f). In all traits except HGT and LN, significant Species × Site interaction terms indicated that *A. arifolium* and *H. nobilis* LA, SLA, LNG and SM responded differently at each location, namely, CWT and WHF. The species also differed by aspect for HGT and by shade treatment for LN.

Mean species LA increased 3.72 cm^2 with watering and was 4 cm^2 greater on north- than south-facing slopes in 2007 (Table 1, a). The significant Species × Site term indicated that *H. nobilis* and *A. arifolium* responded differently to each site as mean *H. nobilis* LA was 4.52 cm^2 greater at WHF than CWT, whereas *A. arifolium* LA was 1.83 cm^2 lower. Mean species SLA was 5.39 $\text{cm}^2\ \text{g}^{-1}$ greater on north-facing slopes (Table 1, b). The significant Species × Site term indicated that mean *A. arifolium* SLA was slightly higher (0.49 $\text{cm}^2\ \text{g}^{-1}$) at CWT than at WHF, whereas *H. nobilis* SLA was much greater (8.55 $\text{cm}^2\ \text{g}^{-1}$). Mean species HGT increased 1.02 cm with shading (Table 1, c). The significant Species × Shading interaction term indicated that HGT in *A. arifolium* increased significantly more with shading (1.35 cm) than in *H. nobilis* (0.85 cm). Mean species LN increased by 0.21 leaves with watering and was 0.18 leaves lower on south-facing slopes (Table 1, d). The significant Species × Shading interaction term indicated that mean *A. arifolium* LN remained unchanged with shading (0.01 leaves), whereas mean *H. nobilis* LN decreased 0.48 leaves with shading. Mean species leaf length increased 0.57 cm with shading and 0.36 cm on north-facing slopes (Table 1, e). The significant Species × Site interaction term indicated that mean *A. arifolium* LNG was 0.54 cm greater at CWT than at WHF, whereas *H. nobilis* LNG was 0.22 cm lower at CWT than at WHF. Mean SM increased 0.03 g with watering and 0.03 g on north- than south-facing slopes (Table 1, f). The significant Species × Site interaction term indicated that mean *A. arifolium* SM was 0.02 g greater at CWT than at WHF, whereas *H. nobilis* SM was 0.06 g lower at CWT than at WHF

Table 1: **analysis of variance (ANOVA) of 2007 trait values by species (*Asarum arifolium*, *Hepatica nobilis*), watering (+,–), shading (+,–), site (Coweeta Hydrologic Laboratory, Whitehall Forest) and slope aspect (North, South)**

Trait[†]	Factor	*MSE*	*F*	*P*
a. LA	Species	6046.5	44.67	<0.01
	Water	1357.9	10.30	<0.01
	Shade	225.4	1.66	0.19
	Site	202.9	1.46	0.22
	Aspect	211.3	15.59	<0.01
	Species × Site	1308.2	9.66	<0.01
b. SLA	Species	39598.0	95.11	<0.01
	Water	559.0	1.34	0.24
	Shade	49.0	0.11	0.73
	Site	2531.0	6.07	0.01
	Aspect	3267.0	7.84	0.01
	Species × Site	1884.0	4.52	0.03
c. HGT	Species	1336.2	487.8	<0.01
	Water	0.6	0.21	0.64
	Shade	152.4	55.62	<0.01
	Site	0.6	0.22	0.62
	Aspect	41.7	15.22	<0.01
	Species × Aspect	15.9	5.81	0.02
d. LN	Species	781.7	404.4	<0.01
	Water	6.9	3.58	0.06
	Shade	7.1	3.67	0.06
	Site	5.2	2.68	0.11
	Aspect	6.1	3.16	0.08
	Species × Shade	8.4	4.32	0.04
e. LNG	Species	10655.2	82.14	<0.01
	Water	3151.9	24.30	<0.01
	Shade	213.5	1.64	0.20
	Site	331.5	2.55	0.11
	Aspect	1698.2	13.09	<0.01
	Species × Site	1920.2	14.80	<0.01
f. SM	Species	0.113	10.29	<0.01
	Water	0.088	8.04	<0.01
	Shade	0.022	2.07	0.15
	Site	0.053	4.82	0.03
	Aspect	0.085	7.78	0.01
	Species × Site	0.175	15.96	<0.01

All Species × Treatment interactions were evaluated using AIC selection and only the strongest interaction for each ANOVA model was retained for evaluation.

[†] Leaf traits: LA = leaf area; SLA = specific leaf area; HGT = plant height; LN = leaf number; LNG = leaf length; SM = shoot mass.

Most of the species trait differences disappeared by 2009 (Fig. 1a–f), as *A. arifolium* only had significantly higher HGT and LN than *H. nobilis* (Table 2, a–f). The LA for both species dropped significantly between 2007 and 2009, *H. nobilis* so much so that the mean difference (±90% CI) between species shrank from 6.9 ± 2.1 to 2.3 ± 4.1 cm^2 (Fig. 1a). Similarly, the mean difference in SLA dropped from 17.7 ± 3.7 to 7.2 ± 12.6 $\text{cm}^2\ \text{g}^{-1}$ between years (Fig. 1b), the mean difference in LNG dropped from 0.9 ± 0.2 to 0.3 ± 0.4 cm (Fig. 1e) and the mean difference in SM dropped from 0.03 ± 0.02 to 0.01 ± 0.03 g (Fig. 1f). The mean HGT of both species dropped significantly between years, but they maintained their mean difference in height from 2007 (3.0 ± 0.3 cm) to 2009 (2.1 ± 0.8 cm; Fig. 1c). The mean number of *H. nobilis* leaves decreased significantly between 2007 and 2009, but *H. nobilis* maintained significantly more leaves than *A. arifolium* in 2007 (2.4 ± 0.3) and 2009 (1.3 ± 0.2; Fig. 1d). Only LA and SLA exhibited significant Species × Aspect interaction, indicating

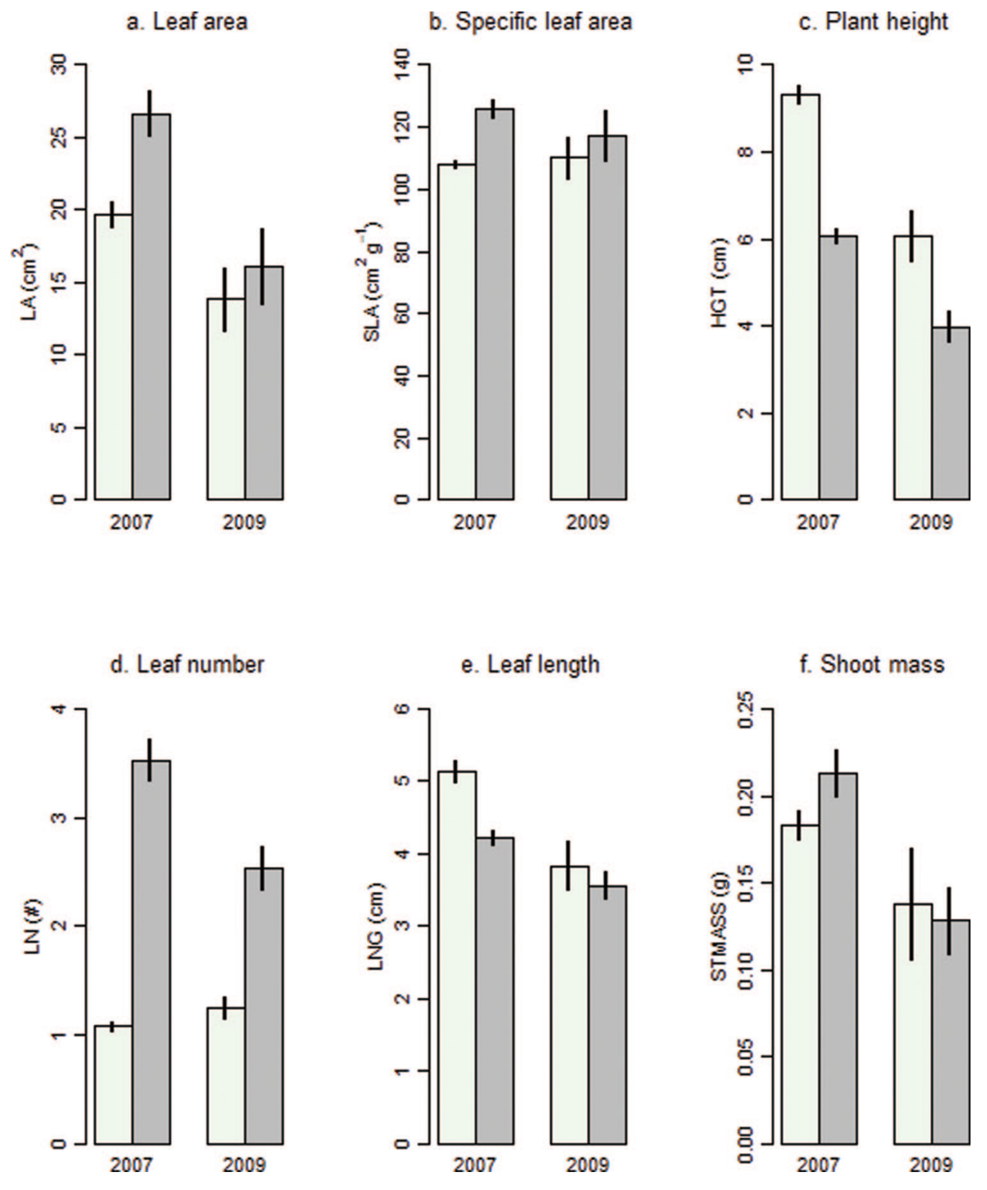

Figure 1: functional leaf traits (mean ± 90% CI) in 2007 and 2009 for *Asarum arifolium* (light-colored bars) and *Hepatica nobilis* (dark-colored bars).

that *A. arifolium* and *H. nobilis* responded differently across slope aspects.

The significant LA Species × Aspect term indicated that, whereas both species responded similarly to the slope aspect, *H. nobilis* LA was much larger (21.37 cm^2) on north- than south-facing slopes than was *A. arifolium* LA (5.53 cm^2) on north- than south-facing slopes (Table 2, a). Similarly, the significant SLA Species × Aspect term indicated that SLA was much larger (41.30 cm^2 g^{-1}) on north- than south-facing slopes in *H. nobilis* than was SLA (18.29 cm^2 g^{-1}) in *A. arifolium* on north- than south-facing slopes (Table 2, b). Mean species HGT was 1.33 cm higher on north- than south-facing slopes (Table 2, c). Mean species LN was 0.30 leaves greater at CWT than WHF and 0.43 leaves greater on north- than south-facing slopes (Table 2, d). Mean species LNG was 0.72 cm greater on north- than south-facing slopes (Table 2, e). Mean species SM was 0.3 g greater on north- than south-facing slopes (Table 2, f).

Relative trait plasticity (±90% CI) across treatments, slope aspect, sites and years was overwhelmingly higher for *H. nobilis* than *A. arifolium* (Figs. 2–4). In general. for both species, plasticity was highest in LA, LN and SM. With watering, mean (± SD) *H. nobilis* plasticity for all traits (22 ± 11%) was higher than in *A. arifolium* (13 ± 8%), particularly with significantly higher LA, SLA, HGT, LN and SM plasticity (Fig. 2a). With shading, *H. nobilis* plasticity for all traits (20 ± 10%) was higher than in *A. arifolium* (14 ± 7%), particularly with significantly higher LA, SLA, LN and SM plasticity (Fig. 2b). The patterns of trait

Table 2: analysis of variance (ANOVA) of 2009 trait values by species (*Asarum arifolium*, *Hepatica nobilis*), site (Coweeta Hydrologic Laboratory, Whitehall Forest) and slope aspect (North, South)

Trait†	Factor	*MSE*	*F*	*P*
a. LA	Species	168.1	1.33	0.25
	Site	172.1	1.36	0.24
	Aspect	2680.1	21.29	<0.01
	Species × Aspect	348.1	2.76	0.09
b. SLA	Species	1614.7	1.38	0.24
	Site	185.1	0.15	0.69
	Aspect	31334.8	26.81	<0.01
	Species × Aspect	4101.7	3.51	0.06
c. HGT	Species	146.6	31.8	<0.01
	Site	9.7	2.11	0.62
	Aspect	48.8	10.60	<0.01
d. LN	Species	81.4	82.5	<0.01
	Site	3.4	3.39	0.06
	Aspect	8.4	8.47	<0.01
	Species × Shade	2.2	2.26	0.13
e. LNG	Species	250.1	1.71	0.19
	Site	67.7	0.46	0.49
	Aspect	1718.1	11.71	<0.01
f. SM	Species	0.003	0.21	0.64
	Site	0.037	2.51	0.11
	Aspect	0.086	5.92	0.02

All Species × Treatment interactions were evaluated using AIC selection and only the strongest interaction for each ANOVA model was retained for evaluation.

†Leaf traits: LA = leaf area; SLA = specific leaf area; HGT = plant height; LN = leaf number; LNG = leaf length; SM = shoot mass.

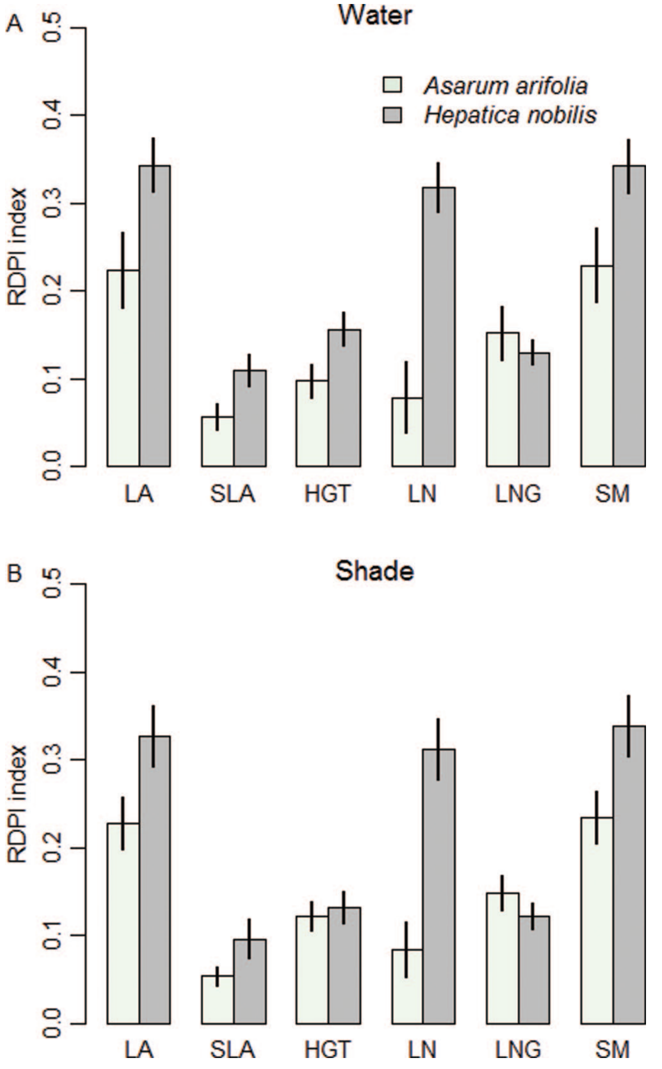

Figure 2: functional trait plasticity (mean ± 90% CI) across watering (A, ±irrigation) and shade (B, ±shadecloth) treatments in 2007 for *Asarum arifolium* (light-colored bars) and *Hepatica nobilis* (dark-colored bars). The traits shown are leaf area (LA), specific leaf area (SLA), plant height (HGT), leaf number (LN), leaf length (LNG) and shoot mass (SM). The relative distance plasticity index (RDPI) is calculated as the absolute value of the difference between trait values of randomized individuals divided by the sum of the paired trait values, resulting in a potential range from zero (no plasticity) to one (maximum plasticity).

plasticity were similar across slope aspect and site to plasticity for experimental treatments (Figs. 2, 3). Mean trait plasticity across slope aspects was higher in *H. nobilis* (22±10%) than in *A. arifolium* (14±7%) for all traits except LNG (Fig. 3a). For variation across transplant sites, *H. nobilis* plasticity (23±12%) also was higher than in *A. arifolium* (15±7%) for all traits except HGT and LNG (Fig. 3b).

In 2009, *H. nobilis* generally remained more plastic than *A. arifolium* across slope aspect and sites, but not as consistently as in 2007. Mean trait plasticity across slope aspects was higher in *H. nobilis* (25±8%) than in *A. arifolium* (14±5%), particularly for LA, HGT and SM (Fig. 4a). Across sites in 2009 was the only situation where mean *H. nobilis* plasticity (28±10%) was not considerably higher than *A. arifolium* plasticity (26±10%), as it was only higher in LN and SM and lower in LNG (Fig. 4b)

DISCUSSION

Trait aggregation along natural environmental gradients may be attributable to phenotypic plasticity by which plants acclimate to environmental heterogeneity (Bradshaw 1965; Rozendaal *et al.* 2006). We translocated two shade-tolerant understory evergreen herbs in environmentally heterogeneous habits and asked whether variation in functional traits corresponds with variation in experimental treatments and environmental gradients. Most of the trait values initially differed between *A. arifolium* and *H. nobilis* and variation in the traits corresponds with variation in environmental conditions, particularly shifts in slope aspect from north to south (Tables 1 and 2; Fig. 1). Because we used two understory evergreen species, a guild whose phenotypic plasticity is considered relatively conserved when compared to other plant types (Grime and Mackey 2002; Reich *et al.* 1992, 1997; Skillman *et al.* 1996;

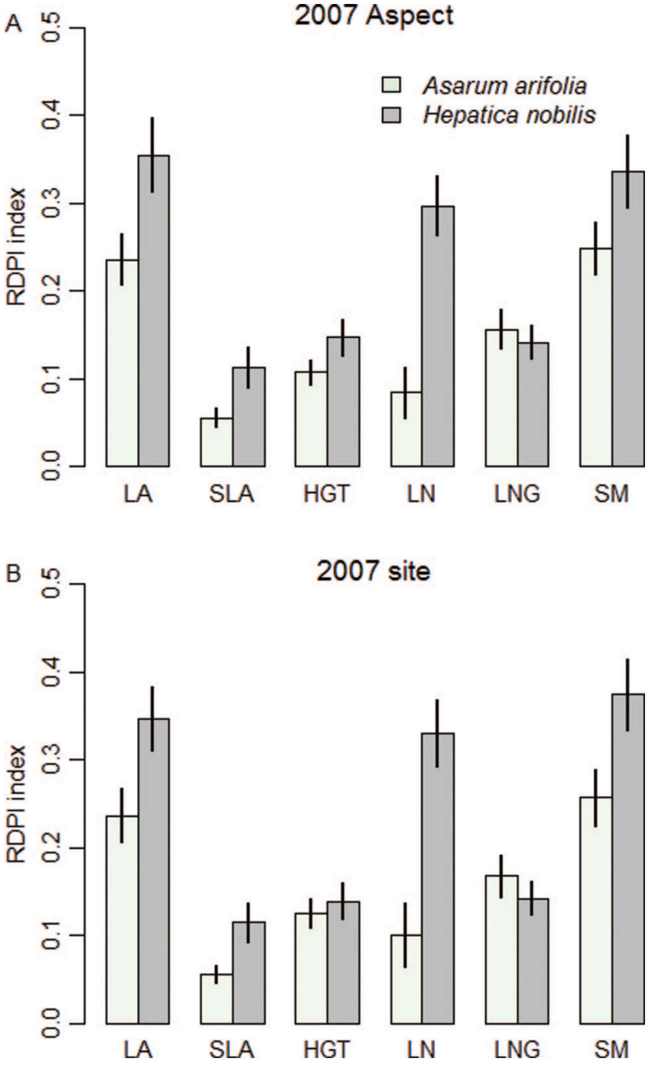

Figure 3: functional trait plasticity (mean ± 90% CI) across slope aspect (A, north/south) and site (B, Coweeta Hydrologic Laboratory/Whitehall Forest) locations in 2007 for *Asarum arifolium* (light-colored bars) and *Hepatica nobilis* (dark-colored bars).

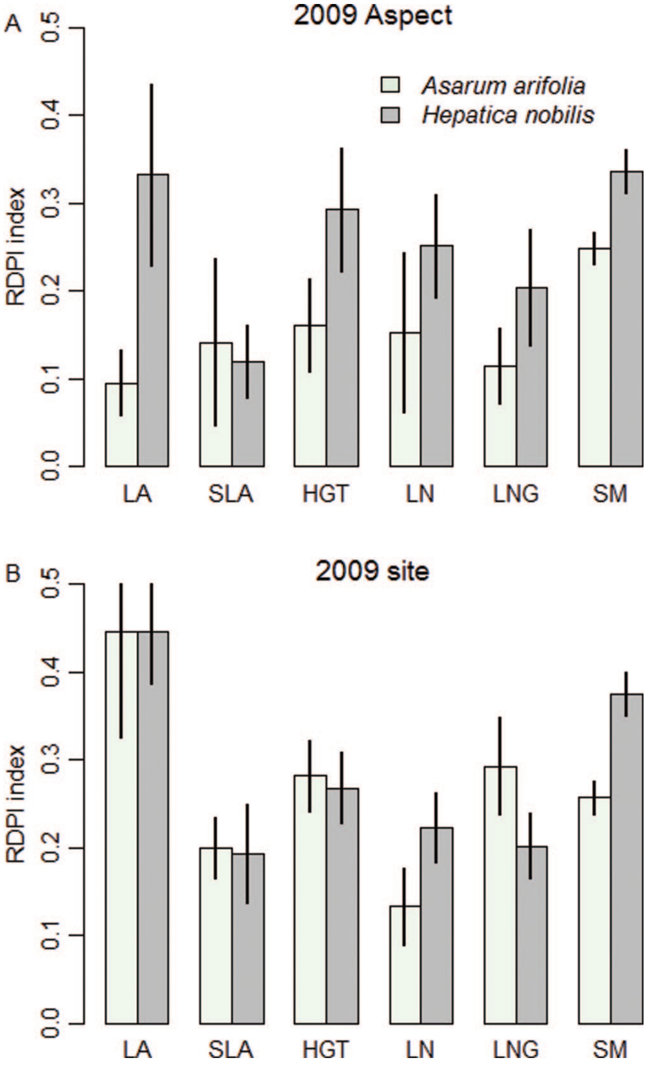

Figure 4: functional trait plasticity (mean ± 90% CI) across slope aspect (A, north/south) and site (B, Coweeta Hydrologic Laboratory/Whitehall Forest) locations in 2009 for *Asarum arifolium* (light-colored bars) and *Hepatica nobilis* (dark-colored bars).

Westoby *et al.* 2002; Wright *et al.* 2004), we expected to find inherent trait values associated with *H. nobilis,* which explain its better survival in the experimental habitats. Alternately, we found a convergence of trait values between species more consistent with phenotypic plasticity. That is, when translocated into novel habitats, and often relatively adverse environmental conditions, individuals of both species adjusted morphologically rather than remaining static and perishing. Moreover, the species that better survives in adverse habitats, *H. nobilis,* consistently exhibits greater phenotypic plasticity.

Species-specific functional trait values and responses

Asarum arifolium and *H. nobilis* are ecologically similar in that they are both shade-tolerant evergreen herbs. However, they are phylogenetically distinct with dissimilar vegetative and reproductive morphology (Appendix 1), pollination biology and distribution. When transplanted into shared plots, all traits differed significantly between species during the first year (Fig. 1a–f). By 2009, however, only HGT and LN differed. The HGT of both species dropped ~3 cm between 2007 and 2009, but *A. arifolium* remained taller in both years. Both plants responded to experimental shading in 2007 with significant increases in HGT (Table 1, c). This is a common reaction in plants as shade (specifically, the red:far-red light ratio associated with shade) prompts stem elongation, presumably so plants can clear obstructions such as accumulated forest floor leaf litter and other plants to enhance light capture (Hicks and Frank 1984; Schmitt and Wulff 1993; Smith 1982). Plant height can convey competitive advantage for light interception (Chu *et al.* 2009; Givnish 1982; Neufeld and Young 2003; Westoby *et al.* 2002), but becomes progressively more costly in terms of biomass investment (see Neufeld and Young 2003), though in *A. arifolium* and *H. nobilis,* shading does not correspond with greater aboveground biomass (Table 1, f). The study species also maintained distinct LN across years as *A. arifolium* rarely produces >1 leaf, whereas *H. nobilis* generally produces 3–4 leaves (Fig. 1d). Despite a distinct difference in LN, both species converge to the same LA in 2009. Thus, each species reaches the same leaf coverage and potential for light interception by producing very different numbers of leaves. Whereas *A. arifolium* and *H. nobilis* LA values converge between years, the overall LA drops for both species (Fig. 2a). The LA for both species decreases significantly with increases in light (Table 2, a), a common LA response (Reich *et al*. 1997). A reduction in LA may reflect an adjustment to decrease boundary-layer resistance and shed excess heat (Cornelissen *et al*. 2003; Evans and Poorter 2001; Parkhurst and Loucks 1972; Rozendaal *et al*. 2006), as well as reduce desiccation (Lambers *et al*. 1998). Given that the study design moved half of each translocated species from presumably benign/tolerable conditions (Warren 2010; Warren and Bradford 2011) to south-facing plots where they would not typically occur (Warren 2008) due to relatively higher temperatures and lower soil moisture (Cantlon 1953; Geiger *et al*. 2003), stress responses by these plants probably results in decreased LA. Indeed, mean LA on south-facing slopes (10.0 cm^2) is approximately half that on north-facing slopes (19.3 cm^2). For understory herbs, particularly evergreens, diminished height also protects against stressful conditions (Givnish 1982), and the plants are somewhat shorter on south- (4.0 cm) than north-facing (5.4 cm) slopes.

The most dramatic convergence in trait values across years occurs in SLA (Fig. 2b). A 2% increase in *A. arifolium* SLA and a 7% decrease in *H. nobilis* SLA between 2007 and 2009 result in statistically identical SLA between species. Mean SLA has been correlated across species with temperature, light and moisture, and low SLA often corresponds with increased plant investment in leaf defenses (i.e. thicker, tougher leaves) and higher light (Ackerly *et al*. 2002; Evans and Poorter 2001; Reich, Ellsworth *et al*. 1998; Wright *et al*. 2004). SLA is considered a key functional marker for plant performance as lower SLA often correlates with a drop in mass-based leaf nutrients and photosynthetic rates (Reich *et al*. 1997; Wright *et al*. 2004). The thick, dense leaves of evergreens (particularly herbaceous species) generally fall on the low end of the SLA spectrum across all plant types, (except for some conifers), reflecting a trade-off favoring persistence over rapid growth (Poorter and Bongers 2006; Reich *et al*. 2003). Even where evergreen trees contradict this pattern (e.g. boreal forests), the evergreen life history appears adaptive for tolerating nutrient-poor conditions (Givnish 2002). Low SLA may give evergreens an advantage in low-resource environments, where persistence and the sequestration of resources outweigh any benefits of fast, competitive growth and disadvantage of greater initial energy investments in leaf structure and limited photosynthetic rates (Neufeld and Young 2003; Reich *et al*. 2003). Thus, the trade-off for the tough, persistent evergreen leaves is an inherent physiological ceiling on maximal photosynthetic rates, limited phenotypic plasticity and consequently low growth rates (Reich *et al*. 1992; Skillman *et al*. 1996). Interestingly, mean SLA for the higher surviving plant, *H. nobilis*, decreases between 2007 to 2009 to match that of the lower surviving plant, *A. arifolium*, which maintained the same mean SLA between years (Fig. 1b), suggesting that phenotypic plasticity, rather than mortality, better explains the observed trends in leaf trait morphology.

Species-specific trait plasticity

Soil moisture, solar irradiance and temperature patterns are very heterogeneous beneath the canopy of deciduous forests (Kato and Komiyama 2002; Neufeld and Young 2003; Warren 2010), and plants must acclimate to persist and thrive (Bradshaw 1965; Grubb 1998; Rozendaal *et al*. 2006; Sims *et al*. 1994). Phenotypic plasticity in leaf and root structure is a means by which plants can maximize foraging of resources, as well as tolerate suboptimal conditions. We observe that *H. nobilis* generally demonstrates greater spatial and temporal plasticity than *A. arifolium* (Figs. 2–4). The higher survival and phenotypic plasticity of *H. nobilis* transplants across abiotic gradients and experimental treatments suggests it is better able to adjust to the environmental extremes. For plants, the capacity to exhibit plastic responses to heterogeneous environments (both spatial and temporal) probably influences how much of the environment the organism can inhabit (Bradshaw 1965; Schlichting and Pigliucci 1995; Valladares *et al*. 2007). The greater phenotypic plasticity in *H. nobilis* than in *A. arifolium* may help explain the discrepancy in the species' distributions as *A. arifolium* is limited to the southeastern region of USA whereas *H. nobilis* spans eastern North America, Europe and Asia.

Leaf traits with high plasticity are presumed more integral for plant functioning in response to an environmental gradient (Bongers and Popma 1988; Rozendaal *et al*. 2006). Shade-tolerant, understory species might be expected to have greater leaf plasticity in order to harvest light in places where it is scarce (Rozendaal *et al*. 2006), and Bongers and Popma (1988) suggest that high plasticity in leaf traits is critical for leaf functioning in different light environments. Both study species exhibit the greatest relative plasticity in LA and SM, but in 2007, *H. nobilis* exhibits much greater plasticity in all traits, except HGT, with variation in watering, shading, slope aspect and transplant site (Figs. 2–3). At the same time, *H. nobilis* exhibited far greater ability to survive adverse transplant conditions, particularly in plots without watering and in south-facing slopes (Appendix 2). Interestingly, the experimental watering and shading treatments do not correspond with greater trait plasticity than slope aspect and site in 2007 (Figs. 2–3), and thus continued evaluation of trait plasticity into 2009 only required the latter (Fig. 4). In 2009, *H. nobilis* remained more plastic across slope aspects, particularly in LA, HGT and SM

(Fig. 4) and continued to outperform *A. arifolium* on the more difficult south-facing slopes (Appendix 2). Neither species exhibited greater survival by site in 2007, but *H. nobilis* survived somewhat better at CWT than at WHF by 2009 and no longer outperforms *A. arifolium* at WHF. Similarly, each species exhibits very little overall difference in trait plasticity across sites in 2009 (Fig. 4), except that *H. nobilis* has greater LN and SM plasticity and *A. arifolium* exhibits greater LNG plasticity. These results suggest that whereas greater trait plasticity in *H. nobilis* gives it the ability to survive in a much wider range of conditions than *A. arifolium*, something mutes this advantage at WHF, where both species exhibit similar longer-term survival.

CONCLUSIONS

We observe an ecological convergence in trait values along environmental gradients between phylogenetically distinct, but ecologically similar, understory evergreen herbs, which is more consistent with phenotypic plasticity than selective mortality. That is, individuals with maladaptive traits appear to adjust leaf morphology to local conditions, particularly *H. nobilis*, rather than die. This exemplifies a remarkable amount of flexibility for a plant guild expected to be relatively unresponsive to environmental heterogeneity. The convergence of leaf traits between species when translocated along environmental gradients suggests that measures of mean values of the leaf traits explored here may be inadequate measures of niche, particularly for core traits such as specific leaf area. Instead, we find that trait phenotypic plasticity better corresponds with the observed performance and natural distributions of the study species. Indeed, the relative spatial and temporal phenotype plasticity that *H. nobilis* exhibits appears to be one of the key differences from *A. arifolium*. The convergence in leaf trait morphology along the experimental gradients obscures absolute niche differentiation but exemplifies a greater capacity to tolerate novel environments and, as a consequence, greater ecological niche breadth.

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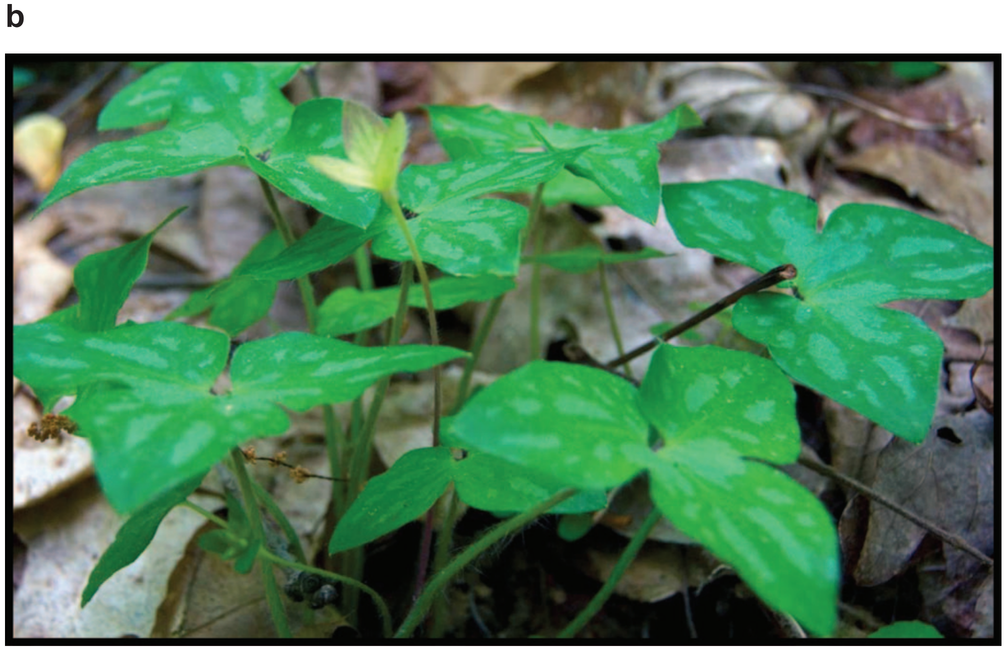
Conflict of interest statement. None declared.

APPENDIX 1

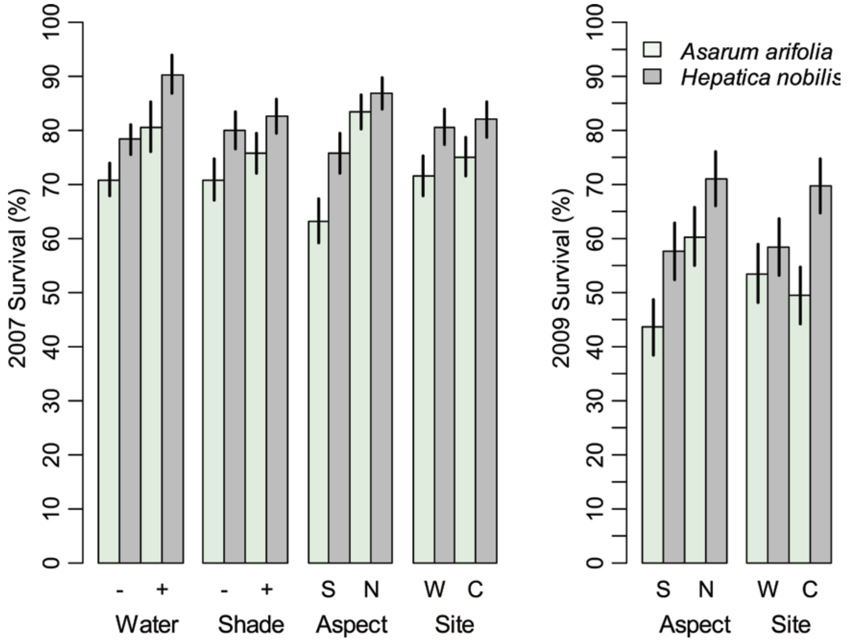
a

Appendix 1 (continued)



Plants of *Asarum arifolium* (a) and *Hepatica nobilis* (b) with seeds set. *Asarum arifolium* seeds are contained in a starchy pod at ground level, whereas *H. nobilis* seeds are clustered at the end of an upright flower stalk. *Asarum arifolium* commonly produces one leaf, whereas *H. nobilis* produces multiple leaves (photos by R. Warren).

APPENDIX 2



Survival of *Asarum arifolium* (light-colored bars) and *Hepatica nobilis* (dark-colored bars) in 2007 and 2009 (these data are presented in a different format in Warren 2010). The watering and shading treatments ("–" = no treatment, "+" = treatment) were discontinued between 2007 and 2009. The transplant locations varied by slope aspect ("S" = south facing; "N" = north facing) and site ("W" = Whitehall Forest, Athens, GA, USA; "C" = Coweeta Hydrologic Laboratory, Otto, NC, USA). Source: Warren RJ (2010).

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