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Ant-mediated seed dispersal does not facilitate niche expansion

Robert J. Warren II[1]*, Itamar Giladi[2] and Mark A. Bradford[1]

[1]*School of Forestry and Environmental Studies, Yale University, New Haven, CT 06511, USA;* [2]*Department of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel*

Summary

1. Whereas classic niche theory is based on the contraction of the niche via negative interactions, facilitative niche theory suggests that mutualisms can expand the niche via positive interactions. Specifically, animal-mediated seed dispersal can expand the utilization of physical space by plants and allow greater access to resources and other environmental requirements. Ant-mediated dispersal of plant propagules (myrmecochory) is a common mutualism throughout the world, particularly in the deciduous forests of the eastern United States where this research is conducted.

2. We examine two facets of niche expansion via ant-mediated seed dispersal: (1) increased utilization of resources along resource gradients and (2) escape from unfavourable density-dependent conditions.

3. We test these assumptions by introducing *Hexastylis arifolia* seeds in cafeteria-style bait stations along abiotic gradients (moisture, temperature, light) for removal by key seed dispersers from the ant genus *Aphaenogaster*. We also examine plant aggregation along the same gradients.

4. Ant-mediated dispersal services decrease significantly with increasing soil moisture and ultimately fail at levels that are demonstratively within the plant's niche optima; further, the decline in dispersal services is correlated with increasing plant aggregation, suggesting that enemy escape also falters at relatively high levels of soil moisture.

5. *Synthesis.* Facilitated propagule dispersal fails to expand the *Hexastylis arifolia* niche in either enhanced resource utilization or decreased density dependence as the niche requirements for the ant disperser are nested within those for the plant. The strength of this interaction varies across space and time, and in doing so may undermine attempts to predict future distributions. Further, given that myrmecochores are typically poor dispersers, the incomplete niche overlap between the plant and its facilitator makes this plant guild particularly susceptible to climatic change if each participant responds individually to shifting environmental conditions.

Key-words: *Aphaenogaster* sp., biotic interactions, dispersal, facilitation, fundamental niche, *Hexastylis arifolia*, physiological limitation, realized niche, species distribution

Introduction

That a superior competitor (or predator) can suppress a species where it would otherwise flourish is a long-established precept in niche theory (Hutchinson 1957; MacArthur & Levins 1967). Hutchinson (1957) provided a simplified framework for this dynamic by delineating a species' environmental requirements as its 'fundamental niche' and the subset of environmental requirements it can actually access in the presence of a superior competitor as its 'realized niche'. Inherently, then, negative biotic interactions reduce the realized niche to a subset of the

fundamental niche (Hutchinson 1957; Pulliam 2000). This approach indelibly links niche theory and competition, but recent work has focused on reshaping the niche concept to include positive interactions also as determinants of the realized niche (Bruno, Stachowicz & Bertness 2003; Freestone 2006; Brooker *et al.* 2008; Van der Putten 2009). That is, if interspecific competition can restrict access to required resources, interspecific cooperation may augment it (Bronstein, Wilson & Morris 2003). This perspective suggests that cooperation between organisms may have as much influence on niche dynamics as competition (Bronstein, Wilson & Morris 2003; Bruno, Stachowicz & Bertness 2003; Freestone 2006). Indeed, Bruno, Stachowicz & Bertness (2003) proposed giving

*Correspondence author: E-mail: hexastylis@gmail.com

facilitation equal footing with competition in ecological theory based on the potential to expand (rather than contract) the fundamental niche via biotic interactions.

A common, facilitative interaction in many plant communities is animal-mediated seed dispersal, which enables plants to utilize a greater portion of physical space than they would otherwise reach via passive transport (Bruno, Stachowicz & Bertness 2003). Seed dispersal by ants (myrmecochory) is a mutualism that occurs throughout the world with more than 3000 plant species (Beattie & Hughes 2002). The lipid-rich seed appendages (elaiosomes) mainly attract non-granivorous ant species that remove the elaiosomes for use as a larval food source while leaving the seeds intact (Beattie & Culver 1981). In turn, myrmecochory potentially benefits plants by increasing the opportunity to utilize suitable microhabitats. That is, by actively transporting seeds to novel habitats, ant-mediated dispersal allows plants to exploit the full range of available resources, particularly those that are optimal for persistence. This is consistent with the suggestion by Bruno, Stachowicz & Bertness (2003) that, unlike competitors, which reduce the fundamental niche to the realized niche, facilitators can expand the realized niche of facilitated species to that of the fundamental niche. Moreover, if the accumulation of adult plants decreases habitat suitability for seedlings and juveniles due to increased disease and predator densities (Janzen-Connell hypothesis, Connell 1971; Janzen 1970) and/or increased inter-specific competition for resources, the emigration of myrmecochore propagules via ant dispersal potentially alleviates these negative effects. From this perspective, ant-mediated seed dispersal is less about exploiting resources in novel microhabitats than escaping density-dependent burdens in established microhabitats (Handel & Beattie 1990; Gorb & Gorb 2003; Giladi 2006; Ness & Morin 2008).

Giladi (2004) translocated *Hexastylis arifolia*, an evergreen woodland myrmecochore native to the south-eastern United States, from upland deciduous forests into floodplain habitats where it was otherwise absent. The transplant populations exhibited per capita growth rates (survival, growth, reproduction) similar to those of populations found in source habitats (deciduous mesic forest). Concurrently, Giladi (2004) found that 49.5% of introduced *H. arifolia* seeds were dispersed by ants in the source habitat whereas only 3.5% were removed in the floodplain. These results suggested the plants were limited from potentially suitable habitat due to failed ant-mediated dispersal, but periodic flooding also caused aborted flowering in adults and poor performance in seedlings in the floodplain habitats (Giladi 2004). This left it questionable whether floodplains were suitable long-term habitat regardless of limitations related to ant-mediated dispersal. Warren (2010) further examined the *H. arifolia* soil moisture response by translocating adults and seeds into deciduous forest habitats not subject to flooding. Adult transplants survived and grew best where soil moisture was highest (amplified via regular watering, see Fig. S1a,b in Supporting Information). Seed recruitment also remained successful in relatively high soil moisture conditions (Fig. S1c). These results indicate that highly mesic conditions

(in un-flooded habitat) can provide suitable habitat for *H. arifolia*.

Zelikova (2008) found a marked increase in *H. arifolia* plant aggregation due to the germination of undispersed seeds near reproductive parents when ants were denied access to naturally released seeds. These findings, along with observations that plant aggregation increases near streams and wet areas (Warren, *pers. obs.*), prompted us to ask whether the limited distribution and aggregation of *H. arifolia* at high soil moisture levels is due to inefficient ant-mediated dispersal. To address this question, we examined ant-mediated seed dispersal along environmental gradients in deciduous forest habitats in the eastern United States where 30–40% of forest herbaceous species are ant-dispersed (Beattie & Culver 1981; Handel, Fisch & Schatz 1981).

In the context of facilitation and niche theory, we tested whether ant-mediated dispersal services occur within or extend beyond the fundamental soil moisture niche of a dominant myrmecochore. That is, we asked whether facilitative seed dispersal occurs throughout the range of soil moistures where the facilitated plant demonstratively can persist (fundamental niche, *sensu* Hutchinson 1957). Ant-mediated seed dispersal may also provide benefit by reducing parent–offspring conflict and among-sibling competition, so that decreased plant aggregation coincides with increased fitness for plants (Janzen 1970; Connell 1971; Howe & Smallwood 1982; Herrera 2002), including myrmecochores (Anderson 1988; Higashi *et al.* 1989; Boyd 2001; Giladi 2004, 2006). If plant aggregation is indeed a product of failed dispersal services, and ant-mediated seed dispersal decreases with soil moisture, this exemplifies a second mechanism by which facilitated dispersal may fail to expand the plant's realized niche. Therefore, in addition to seed dispersal rates, we also investigated whether ant-facilitated dispersal services are associated with *H. arifolia* plant aggregation along the soil moisture gradients.

Materials and methods

STUDY SPECIES AND SITES

Hexastylis arifolia (Michx.) Small (Aristolochiaceae) is a short (7–12 cm), perennial, understorey, evergreen herb that occurs in the temperate deciduous forests of the south-eastern United States, and it produces propagules that are distributed by ants (Zelikova, Dunn & Sanders 2008). We selected two sites within 100 km of each other, both dominated by the mature (60–80 years), deciduous forests (oak–maple–hickory) characteristic of the region. The first site (MO) was located in the Middle Oconee River watershed within a natural area of the State Botanical Garden of Georgia, Clarke County, Georgia, USA (35°54′08″N, 83°22′58 ″W, 190–210 m a.s.l., 240–290° aspect, 0–31° slope). This site is located near those used by Giladi (2004), Zelikova (2008) and Warren (2010). The second site was in the Betty's Creek (BC) watershed in the Cowee Mountains of Jackson County, North Carolina, USA (35°17′10″N, 85°17′27″W, 777–790 m a.s.l., 310–360° aspect, 9–44° slope). Although *H. arifolia* occurs in the vicinities of both sites, only at the MO site was *H. arifolia* immediately present. This second site, which is found within the wetter, cooler climate in the southern Appalachian Mountains, was included in our

study to extend the representation of ambient environmental gradients in our data and, in so doing, increase the generalizability of results.

SEED REMOVAL RATES

In May 2008, 1000 mature *H. arifolia* seeds were collected in the vicinity of each site and stored in plastic vials at 4.5 °C for use within 3 days or at −2 °C for use within 10 days; neither storage method appears to have an effect on seed attraction for ants (Ness & Morin 2008). Next, in each site we established five 20-m transects, perpendicular to a small stream, at *c.* 100-m intervals. Along each transect, at 5-m intervals, we established 2-m^2 plots giving a total of 25 plots per site. At the centre of each plot, we placed a bait station (Fig. S2) that consisted of a 4 × 4 cm polystyrene weighing dish secured into the soil with a 2-cm nail. At each station, 10 seeds were introduced and surveyed at 1-h intervals for 4 h to measure removal rates by ants. The length of seed introduction intervals used here meet or exceed those typical for myrmecochory research as most seed removal by ants occurs within the first 1–2 h of seed release (Gorb & Gorb 2003; Heithaus & Humes 2003; Heithaus, Heithaus & Liu 2005; Ness, Morin & Giladi 2009). Four trials were conducted at each station for a total of 40 seeds per station, 200 seeds per transect, 1000 seeds per site. The work was repeated in May 2009 (giving $n = 4000$ seeds total).

All seed introduction trials were conducted between 8:00 and 13:00 h on days without precipitation during May–June 2008. These times coincided with the natural period of seed release and peak ant foraging activity (Handel & Beattie 1990; Gorb & Gorb 1998; Giladi 2004). At the MO site, small gastropods were occasionally observed and removed from the bait stations. At the end of each seed introduction trial, the bait stations were used to collect ant specimens (after first calculating removal rates) that actively grasped and removed seeds for species identification. Ant species were identified using Fisher & Cover (2007) and Creighton (1950). We intended to quantify the interaction between myrmecochores and seed-dispersing ants and not the ant community as a whole. The latter would have required additional sampling techniques such as litter extractions and pitfall traps. What our bait stations do provide is a reasonable measure of myrmecochorous ant foraging efficiency (Bestelmeyer *et al.* 2000; Wang, Strazanac & Butler 2001b).

ENVIRONMENTAL VARIABLES

Volumetric soil moisture percentage was measured with a handheld Hydrosense Soil Water Content Measurement System (Campbell Scientific, Inc., Logan, UT, USA) four times in May-June 2008 and 2009; we used the average of five points per plot for further analysis. Percentage diffuse light was calculated from hemispherical photographs (Nikon SLR; Nikon fisheye lens; self-levelling gimbal) using Gap Light Analyzer 2.0 software. To reduce contrast between reflection and true sky and control temporal variation between grids, all photographs were taken at each plot on cloudy days between 10:00 and 14:00 h. Ambient soil temperature was measured in each plot with a T-shaped digital thermometer inserted 8 cm into the soil; we used the average of five points per plot for analysis

PLANT MICROSITE DISTRIBUTION

Hexastylis arifolia individuals were surveyed for abundance (plants m^{-2}), age class (seedling or adult) and reproduction (fruits plant^{-1}) in each plot at the MO site in both 2008 and 2009. The 2-m^2 plot scale was appropriate for capturing the influence of microclimate variation

on both adult and seedling plant abundance, and adult reproduction, but ant-dispersal typically occurs at scales < 1 m^2 (Giladi 2004; Zelikova 2008). For example, without ant-dispersal, *H. arifolia* seeds only travel approximately 2–12 cm from source plants while ant-dispersed seeds travel approximately 9–71 cm (Zelikova 2008). Further, destructive harvesting from prior *H. arifolia* research ($n = 144$) indicated that > 1 leaf plant^{-1} is uncommon and ≥3 leaves plant^{-1} extremely rare (mean leaves per plant 1.2 ± 0.4, median = 1) (Warren 2007). These results strongly indicate that a cluster of ≥3 *H. arifolia* leaves in ≤81 cm^2 is likely the result of multiple plants that germinated from undispersed seeds near maternal plants (Fig. S3). This was repeatedly confirmed by the presence of seedlings within clusters of *H. arifolia* leaves (Warren, pers. obs.). In order to measure plant aggregation, the number of clustered aggregates (≥3 leaves in ≤81 cm^2) were counted per plot and standardized as aggregate units per total abundance. Plots with ≤2 plants were omitted from analysis.

DATA ANALYSIS

The proportion of seeds removed from bait stations by ant dispersers at each site (MO and BC) was analysed with a logistic regression using generalized linear mixed models (GLMMs) assuming binomial error distributions. Soil moisture, diffuse light and temperature, along with interaction terms, were included in the models as fixed effects, and second-order terms were included to account for unimodal responses. Given that the clustering of plots along transects might introduce spatial autocorrelation to the data, transect was included as a random effect. The GLMMs were fit using the Laplace approximation in the 'lme4' package for the 'R' statistical program (R, 2005). Model selection was based on the Akaike information criterion (AIC). Similar 'best' models (AIC < 4) were averaged to determine the relative Akaike weights of the parameters.

The results indicated soil moisture was the main driver behind dispersal services along the gradients, and this prompted us to investigate *H. arifolia* adult and seedling abundance (total plants), reproduction (fruits plant^{-1} and aggregation (clusters total plants^{-1})) as a function of soil moisture gradients at MO. We again employed GLMM models assuming a Poisson error distribution for the adult and seedling abundance and a binomial error distribution for reproduction. We evaluated aggregation as a function of soil moisture using a logistic regression assuming a binomial error distribution for the proportion of clustered plants per all plants in a plot. We also used GLMM models assuming Poisson error distribution to evaluate the correlation between plant abundance and seed removal by ants.

Soil moisture, temperature and diffuse light might be expected to be collinear due to the association of light with temperature, and temperature with evaporation. We checked for potential collinearity and found the variance inflation factors for all the models used here were < 1.5, indicating they independently account for variance. The overdispersion parameter in all models was < 1 – except seedling abundance, which was accounted for by using the quasiPoisson error distribution and quasiAIC model selection.

Two outliers in the 2008 MO data prompted a *post hoc* analysis of seed removal and soil moisture using quantile regression. Quantile regression estimates at the 0.50 interval should match the least-square mean if no significant outliers at the upper extremes influence the estimate. Here, lines fitted to the least-squares residual mean and to the quantile regression 0.50 interval were almost identical at both sites and years, except in 2008 at MO. This may indicate that a severe drought in the MO-site region gave rise to outliers that altered what would have been a typical soil moisture response across those other data collected (see Results and Discussion).

Results

DISPERSER IDENTITY AND ENVIRONMENTAL CONDITIONS

One thousand seeds were presented at each site in each year ($n = 4000$ seeds total) and 22.3% were removed by ants in 2008 (25.5% at MO, 19.1% at BC) and 34.3% in 2009 (29.7% at MO, 38.9% at BC). Mean (±SD) moisture was similar across sites and years (BC 2008 = 22.40 ± 7.2%; BC 2009 = 21.96 ± 5.6%; MO 2009 = 21.36 ± 5.5%) except for MO in 2008 (17.29 ± 4.9%), which reflects a severe regional drought (NOAA, 2009). Mean diffuse light (±SD) was somewhat higher at BC (28.76 ± 4.6%) than MO (23.29 ± 3.6%) while mean temperature (±SD) was higher at MO (19.24 ± 0.2 °C) than BC (17.57 ± 0.5 °C).

Five ant species were identified removing seeds from bait stations: *Aphaenogaster picea* (Wheeler), *Aphaenogaster rudis* Enzmann, *Camponotus pennsylvanicus* (DeGeer), *Formica subsericea* Latreille and *Prenolepis imparis* (Say). *Aphaenogaster* spp. were the most abundant (77% of 91 ants identified); *A. rudis* was found at MO and *A. picea* was found at BC. *Formica subsericea* was the only species observed at both sites, and *C. pennsylvanicus* and *P. imparis* were only found at MO. The *Aphaenogaster* species in this study are common, behaviourally subordinate, individually foraging ants which inhabit transient, shallow nests (Lynch, Balinsky & Vail 1980; Fellers 1989). They specialize in fast discovery of food resources and are quantitatively and qualitatively the most efficient seed dispersing ants in deciduous forests in the eastern United States (Ness, Morin & Giladi 2009). *Prenolepis imparis* is an aggressive, behaviourally dominant small ant that often monopolizes food resources (Lynch, Balinsky & Vail 1980). It usually either ignores myrmecochorous seeds or consumes elaiosomes on site without providing any dispersal services (Giladi 2004; Ness, Morin & Giladi 2009). The other two species (*C. pennsylvanicus* and *F. subsericea*) are larger species which form large colonies, exhibit wide foraging ranges, but which occasionally collect and transport seeds of myrmecochores (Giladi 2004; Ness, Morin & Giladi 2009).

DISPERSAL SERVICES

Factors were selected from the full model (seed removal ~ light + light^2 + temperature + temperature^2 + soil moisture + soil moisture^2 + light × temperature × soil moisture | + transect) to determine the minimum adequate models sufficient for predicting seed removal at MO and BC in 2008 and 2009. The model that adequately explained seed removal by ants from bait stations at MO in 2008 only included temperature as a covariate, and it indicated that seed removal decreased significantly with temperature (coeff. = −2.89, SE = 0.73, $z = -3.97$, $P < 0.001$). However, quantile regression indicated the MO 2008 results were heavily influenced by two outliers and that once the outliers were accounted for, the soil moisture response pattern followed that of the other years and sites (Fig. 1). The outliers are not viewed as error, however, as this

altering of the typical pattern by the outliers in a severe drought has biological significance (see Discussion).

In 2009 at MO, the minimum adequate model included temperature and soil moisture, and it indicated that seed removal decreased significantly with temperature (coeff. = −4.35, SE = 0.61, $z = -7.15$, $P < 0.001$) and soil moisture (coeff = −0.13, SE = 0.02, $z = -6.45$, $P < 0.001$) (Fig. 1b). The minimum adequate model for BC only included a negative relationship with soil moisture in both years (2008: coeff = −0.11, SE = 0.02, $z = -6.323$, $P < 0.001$; 2009: coeff. = −0.24, SE = 0.03, $z = -9.69$, $P < 0.001$) (Fig. 1d).

PLANT ABUNDANCE, REPRODUCTION AND AGGREGATION

In 2008, 111 *H. arifolia* adults and 20 seedlings were surveyed along the MO transects; in 2009 136 adults and 56 seedlings were surveyed. Adult *H. arifolia* abundance (plants m^{-2}) decreased with soil moisture (coeff. = −0.03, SE = 0.02, $z = -1.93$, $P = 0.053$) (Fig. 2a) while seedling abundance peaked at intermediate soil moisture levels (first-order coeff. = 0.76, SE = 0.28, $z = 2.71$, $P = 0.007$; second-order coeff. = −21.08, SE = 7.95, $z = -2.65$, $P = 0.008$) (Fig. 2b). Soil moisture had no significant influence upon reproduction (coeff. = 0.07, SE = 0.01, $z = 14.33$, $P = 0.26$) (Fig. 2c), yet *H. arifolia* aggregation increased with soil moisture (coeff. = 0.14, SE = 0.03, $z = 3.91$, $P < 0.001$) (Fig. 2d).

The influence of myrmecochore abundance on seed removal from bait stations varied by site and year and provided no consistent pattern. At MO, seed removal was negatively correlated with myrmecochore abundance in 2008 (coeff. = −0.06, SE = 0.03, $z = -2.27$, $P > 0.10$) and positively correlated with abundance in 2009 (coeff. = 0.10, SE = 0.02, $z = 4.29$, $P < 0.001$). There was no correlation between seed removal and myrmecochore abundance at BC in 2008 (coeff. = −0.01, SE = 0.009, $z = -1.37$, $P > 0.10$) or 2009 (coeff. = −0.01, SE = 0.009, $z = -1.40$, $P > 0.10$).

Discussion

FACILITATION AND NICHE THEORY

Facilitative niche theory proposes that whereas competitive interactions contract an organism's fundamental niche (inherent environmental requirements) to its realized niche (available environmental requirements not excluded by presence of a superior competitor), the assistance of a mutualist can extend the realized niche to at least the boundaries of the fundamental niche (Bruno, Stachowicz & Bertness 2003). This necessitates that facilitators operate throughout or beyond the fundamental niche requirements of the facilitated species, and where niche requirements overlap, the environmental conditions are unlikely to be optimal for both (Bronstein 1989). The large-scale distribution of myrmecochorous plants and their key dispersers, *Aphaenogaster* spp., shows a remarkable amount of large-scale overlap in the deciduous forests of the eastern United States (Ness, Morin & Giladi
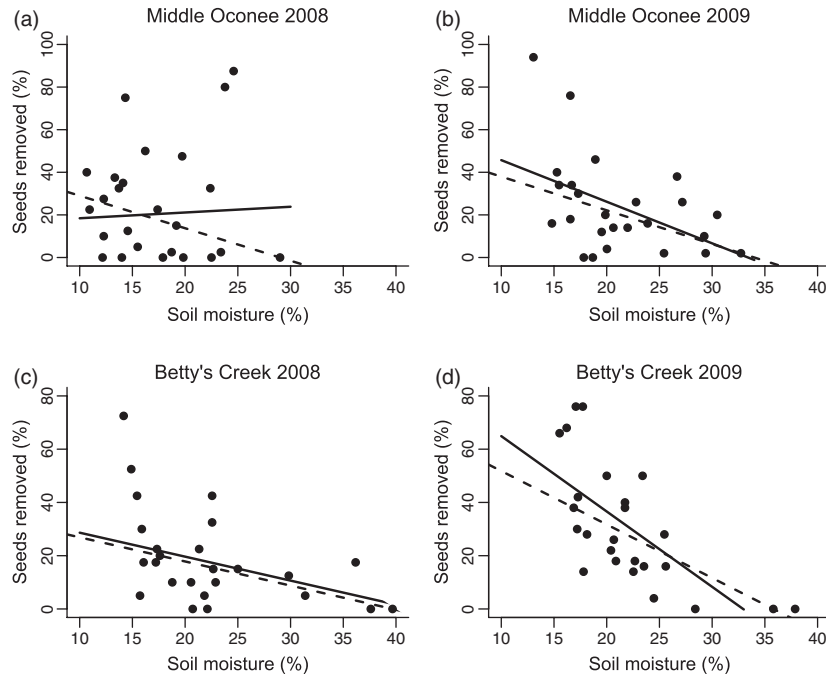
Fig. 1. Ant-mediated dispersal of *Hexastylis arifolia* propagules along soil moisture gradients at the Middle Oconee, Georgia, USA (a, b) and Betty's Creek, North Carolina, USA (c, d) sites in 2008 and 2009. Data points represent the percentage of seeds removed from the total introduced (four trials, $n = 40$) in each plot as a function of mean soil moisture for the duration of seed introduction (April–May). Solid lines indicate fitted residual lines from least-squares regression, and the slope of all except (a) differ significantly from zero. Dashed lines indicate the conditional 50th quartile (median) of the dependent variable. The deviation between the least squares and quantile regression lines in (a) Middle Oconee 2008 data reflects the considerable influence of the outliers on the least squares mean.
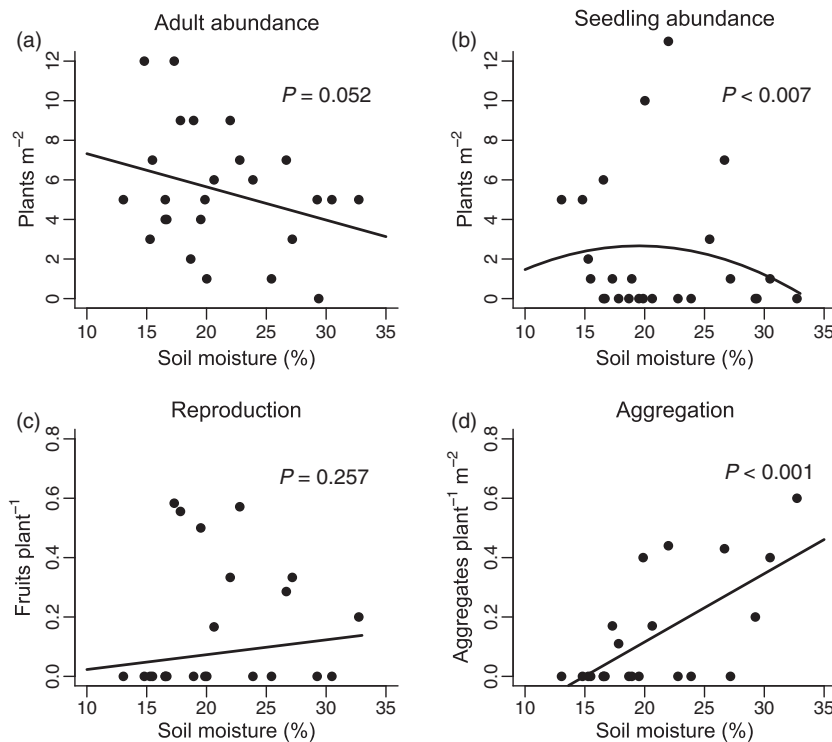
Fig. 2. 2009 *Hexastylis arifolia* adult (a) and seedling (b) abundance, per capita reproduction (c) and aggregation (aggregated clumps as a proportion of all plants in the plot) (d) along soil moisture gradients at the Middle Oconee, GA, USA, site.

2009). We show that this facilitative interaction can fail at finer scales, however, and this can be linked to environmental conditions. Specifically, *Aphaenogaster* spp.-mediated dispersal services approximate zero at higher soil moisture levels (Fig. 1) where *H. arifolia* adults are highly clumped, indicating multiple generations of surviving seedlings

(Fig. 2c, d). Moreover, transplant experiments indicate that *H. arifolia* adults and seedlings survive and grow where soil moisture exceeds the conditions observed in these seed-dispersal experiments (Fig. S1). For example, ant-mediated seed dispersal decreases to 0 where soil moisture is >30%, yet adult transplant survival and growth are highest in such conditions (Fig. S1a,b) and seed recruitment remains relatively steady across soil moisture gradients ranging from 0 to 51%, although it drops somewhat when soil moisture is >41% (Fig. S1c). By failing to facilitate *H. arifolia*'s ability to exploit its full range of niche requirements, the limits of a positive biotic interaction contract the fundamental to the realized niche in a pattern similar to the classic contraction driven by competition or predation. Similarly, Kjellberg & Valdeyron (1990) found that species-specific pollination limited rather than enhanced range expansion of *Ficus carica* due to the niche limits of its pollinator.

Our results do not preclude the possibility that seed dispersal by ants contributes to the expansion of *H. arifolia*'s realized niche at the lower end of the soil moisture gradient or along other environmental gradients. Nevertheless, ant-mediated dispersal may instead most benefit *H. arifolia* by alleviating small-scale density-dependent burdens such as competition and disease (Cain, Damman & Muit 1998; Giladi 2006). We find a correlation between increased seed removal by ants and decreased plant aggregation (consistent with decreased clustering of undispersed seeds around reproductive adults), and this may alleviate localized density-dependent effects (Fig. 2). At higher soil moisture, where the mutualism breaks down, *H. arifolia* seeds remained undispersed and aggregation increases, which is maladaptive (Anderson 1988; Higashi *et al.* 1989; Boyd 2001; Giladi 2004, 2006).

In the southern Appalachian Mountain region (USA), where our work was performed, ant diversity and myrmecochorous seed removal decreases with elevation, putatively as a function of decreasing temperature (Sanders *et al.* 2007; Zelikova, Dunn & Sanders 2008). We also found temperature shifts important in explaining dispersal services at the warmest site (MO), but the decreased seed removal with higher temperatures found here is the inverse of that seen at the elevational gradients where seed removal increases with temperature (Sanders *et al.* 2007; Zelikova, Dunn & Sanders 2008). This may be a function of spatial scale as our investigation occurs along metre transects separated, at most, by 1 km, whereas the elevational research occurred at the scale of kilometres, and thus, more than spatial gradients, these approaches addressed very different temperature gradients. We observed a temperature gradient of approximately 17–20 °C while Sanders *et al.* (2007) observed a gradient of 12–25 °C. Notwithstanding, precipitation also increases with elevation (and evaporation decreases), and the results reported here suggest that soil moisture also must be investigated as a driver of ant-mediated dispersal services. We found no relation between dispersal services and diffuse light. In addition, although the density of inter- or intraspecific myrmecochores is speculated to affect seed removal

rates by ants (Smith, Forman & Boyd 1989b; Smith *et al.* 1989a), we found no evidence that the abundance or identity of background myrmecochores had any confounding impact on seed removal at either site.

The dominant ant dispersers from our bait stations were from the *Aphaenogaster* genus, and this is consistent with a growing body of research suggesting a specialization by this genus in association with myrmecochores (Mitchell, Turner & Pearson 2002; Zelikova, Dunn & Sanders 2008; Ness, Morin & Giladi 2009). Research into the impact of soil moisture on ant behaviour is not common, but Wang, Strazanac & Butler (2001a) found increased soil moisture to be a negative predictor of ant diversity, and Levings (1983) suggested that increased soil moisture reduces ant foraging and decreases the suitability of nest sites. Higher soil moisture also causes increased fungal infections in ant colonies (Clark & Prusso 1986; Chen, Hansen & Brown 2002); and soil inundation from periodic flooding appears calamitous for ground-dwelling ant species (Cogni, Freitas & Oliveira 2003; Ballinger, Lake & Mac Nally 2007). However, it has also been shown that many ant species avoid desiccation (Kaspari 1993; Kaspari & Weiser 2000). Because our research targeted *H. arifolia*, which does not occur in the driest habitats within its range (Warren 2008, 2010), the bait stations may have only captured one end of the ant niche, and the discrepancy between our and other research is simply due to sampling at different points along the gradient (*see* Van Horne 2002).

The soil moisture limit on the ants may occur via nesting restrictions, but, at minimum, the results here indicate it curtails foraging. The increased aggregation of *H. arifolia* plants (Fig. 2d) indicates that myrmecochore propagules occasionally reach the wetter areas of the landscape, possibly by means other than ant dispersal (Fig. 2a), such as gravity and/or overland water flow, and subsequently produce undispersed propagules. An additional possibility is that the plants are dispersed into typically wet habitat during drought conditions. *Aphaenogaster* ants and their colonies are highly mobile and move their nests – found in soil, folded leaves and below rocks or bark – as often as every 30 days (Smallwood 1982; McGlynn *et al.* 2004). As such, the ants may migrate into otherwise wet habitats during drought years. Our finding that the negative relationship between seed removal and soil moisture broke down during the extreme MO drought in 2008 (Fig. 1a) supports this hypothesis. The top three seed removal sites in 2008, during the drought, were not the top three the next year when soil moisture increased. Further, at MO, there is no significant relationship between seed removal per individual plot in the drought year (2008) and subsequent non-drought year (2009) (linear regression: coeff. = 0.263, $t = 1.199$, $P = 0.242$), whereas at BC, where rainfall was similar between years, seed removal per individual plot in 2008 correlated significantly with seed removal per individual plot in 2009 (linear regression: coeff. = 1.113, $t = 4.631$, $P = 0.001$). This suggests that ant-dispersers moved into plots during the drought at MO that they would otherwise avoid in typically wet years.

SPECIES DISTRIBUTIONS AND CLIMATE

Considering linkages between conceptual and practical niche ideas, our data are important for evaluating the likelihood that predictive models of plant distribution will yield reliable forecasts. For example, a criticism of species distribution models is that they fail to incorporate biotic interactions (Davis *et al.* 1998; Araujo & Luoto 2007) – although such models typically make predictions at geographic scales where species are theorized to be more influenced by climate than biotic interactions (Soberon 2007). Indeed, the impact of resource availability on species interactions and the impact of those interactions on populations and communities remain a longstanding theoretical dilemma in ecology. If biotic interactions between species remain constant across resource gradients, mapping the abiotic variables defining the organism's niche will provide a reasonable approximation of potential distributions. However, the nature and intensity of competitive interactions can vary with resource gradients (Leathwick & Austin 2001; Pearson & Dawson 2003), and we show that the intensity of facilitative interactions between ant-dispersers and *H. arifolia* vary along soil moisture and, somewhat, temperature gradients (Fig. 1). Studies of facilitative interactions assessing resource enhancement and habitat amelioration suggest that facilitative interaction strengths vary with abiotic conditions (Maestre, Valladares & Reynolds 2003; Freestone 2006; Maestre *et al.* 2009), and our data on recruitment enhancement suggest this may be a general pattern for facilitative interactions.

If the strength of facilitative interactions changes in a systematic way with an environmental gradient, the inclusion of the environmental parameter as a surrogate for the biotic interactions in predictive models (without explicitly modelling the interactions) should not severely affect the model efficacy. Problems will arise, however, if the facilitative relationships can not be generalized or are not transferable. The similarity in dispersal services as a function of soil moisture between two study sites (MO and BC) located 100 km apart and in different biomes is encouraging for the use of soil moisture as a proxy for the dispersal interaction; however, the increase in dispersal services from 2008 to 2009 in the outlier sites at MO is quite disproportional to the change in soil moisture across years (Fig. 1). This suggests that ant dispersal services may shift in an unpredictable manner during drought years and this makes the omission of this biotic interaction problematic in predicting potential distributions based on current patterns. The strength of ant seed-dispersal services varies with geographic and environmental gradients (Mitchell, Turner & Pearson 2002; Giladi 2004; Kersch & Fonseca 2005; Zelikova, Dunn & Sanders 2008), and reliable niche models for myrmecochores will need to capture ant as well as plant responses to changing climate. This may be especially true given the asymmetry of the mutualism (i.e. the ant is not particularly reliant on the plant for food, yet the plant is predominantly reliant on the ant for dispersal (Kersch & Fonseca 2005; Zelikova, Dunn & Sanders 2008; Ness, Morin & Giladi 2009).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. *Hexastylis arifolia* survival, growth and seedling recruitment across soil moisture gradients in deciduous forest habitat.

Figure S2. Photo of cafeteria-style bait stations offering *Hexastylis arifolia* seeds for removal and dispersal by ants.

Figure S3. Photo of typical and spatially aggregated *Hexastylis arifolia* plants.

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