Phenotypic plasticity despite source–sink population dynamics in a long-lived perennial plant

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Summary

• Species that exhibit adaptive plasticity alter their phenotypes in response to environmental conditions, thereby maximizing fitness in heterogeneous landscapes. However, under demographic source–sink dynamics, selection should favor traits that enhance fitness in the source habitat at the expense of fitness in the marginal habitat. Consistent with source–sink dynamics, the perennial blueberry, Vaccinium elliottii (Ericaceae), shows substantially higher fitness and population sizes in dry upland forests than in flood-prone bottomland forests, and asymmetrical gene flow occurs from upland populations into bottomland populations. Here, we examined whether this species expresses plasticity to these distinct environments despite source–sink dynamics.

• We assessed phenotypic responses to a complex environmental gradient in the field and to water stress in the glasshouse.

• Contrary to expectations, V. elliottii exhibited a high degree of plasticity in foliar and root traits (specific leaf area, carbon isotope ratios, foliar nitrogen content, root : shoot ratio, root porosity and root architecture).

• We propose that plasticity can be maintained in source–sink systems if it is favored within the source habitat and/or a phylogenetic artifact that is not costly. Additionally, plasticity could be advantageous if habitat-based differences in fitness result from incipient niche expansion. Our results illuminate the importance of evaluating phenotypic traits and fitness components across heterogeneous landscapes.

Introduction

Adaptive phenotypic plasticity, where phenotypes shift in response to changing environmental conditions, is a strategy that maximizes fitness across heterogeneous landscapes (Dudley & Schmitt, 1996; van Tienderen, 1997). Adaptive plasticity can evolve when individuals experience multiple environments during their lifetime (fine-grained temporal variation) (Moran, 1992; Stratton & Bennington, 1998), or if progeny establish in a nonparental habitat (fine-grained spatial variation) (Alpert & Simms, 2002). The evolution of adaptive plasticity hinges on the requirements that individuals can adequately track environmental changes, can overcome the costs of a plastic response and are at a fitness advantage if they alter phenotypes in different habitats or in response to different environmental conditions (Via & Lande, 1985; Moran, 1992; van Tienderen, 1997; DeWitt et al., 1998; Alpert & Simms, 2002; Poulton & Winn, 2002; Sultan & Spencer, 2002; Hollander, 2008).

In a spatially variable landscape, the evolution and/or maintenance of phenotypic plasticity can be constrained when fitness is significantly lower in one habitat, as is the case when source–sink dynamics regulate population demography. Source–sink dynamics occur when one habitat is substantially more common or more favorable than another, such that reproductive output in one habitat swamps reproductive output in another habitat and gene flow occurs primarily from the source habitat into the sink.
habitats (Pulliam, 1988). The literature distinguishes between absolute sinks, where no reproduction occurs and the population is maintained solely by immigration, and relative sinks, where reproduction is substantially lower than in the source habitat and the population is supplemented by immigration from the source (e.g. Kawecki, 2008). Theory predicts that selection will favor traits that are advantageous in the higher-quality or larger habitat at the expense of adaptations to the sink habitat, but an absolute or relative sink; a plastic response is unlikely to evolve if populations in one habitat contribute substantially less to the metapopulation gene pool than populations in an alternative habitat (Holt & Gaines, 1992; Moran, 1992; Kawecki, 1995; Sultan & Spencer, 2002; Ernande & Dieckmann, 2004). Selection for plasticity in a spatially heterogeneous landscape with source–sink dynamics should be minimal because a relatively low proportion of the population experiences the sink habitat; furthermore, genotypes specialized to the source habitat would probably outcompete plastic genotypes if there are inherent costs of plasticity (Dias, 1996; Kawecki, 2008). Alternatively, if the costs of plasticity are negligible, or there is limited genetic variation, plasticity could be maintained as a phylogenetic legacy, even if environmental variation in the source habitat was minimal. At the same time, adaptive plasticity could facilitate range or niche expansion (e.g. Schlichting & Smith, 2002) and enhance establishment and persistence in ecologically marginal habitats.

Empirical studies of source–sink dynamics focus on gene flow, population demography and fitness differences between habitats (e.g. Stanton & Galen, 1997; Caudill, 2003), but phenotypic and behavioral traits of individuals in source and sink populations are rarely quantified (but see Caudill & Peckarsky, 2003). Conversely, studies of phenotypic plasticity in spatially variable environments do not often explicitly examine the effects of habitat frequency, or investigate populations that differ in abundance and fitness in alternate habitats (but see Jurjavcic et al., 2001; Huber et al., 2004; Lind & Johansson, 2007; Saunders et al., 2009; Snell-Rood & Papaj, 2009). Vaccinium elliottii (Ericaceae), a species of highbush blueberry, occurs throughout the southeastern USA in seasonally flooded bottomland forests and xeric upland forests (Radford et al., 1968; Godfrey & Wooten, 1981). In a previous study, we documented limited neutral differentiation between populations of V. elliottii in contrasting upland and bottomland habitats ($F_{ST} = 0.03$, Anderson & Geber, 2010), which in addition to extensive inter- and intra-annual variation in flooding in the bottomland habitat, would appear to promote the evolution of plasticity. However, population size was significantly higher, and naturally recruited adults had over an order of magnitude more reproductive structures (flowers and fruits), in upland forests than in bottomland forests, resulting in a reproductive output more than 50 times higher per unit area in upland, relative to bottomland, forests (Anderson & Geber, 2010). Furthermore, experimental individuals transplanted into upland sites had a survivorship 2.5 times higher and growth rates over 1.5 times higher than bottomland transplants (Anderson & Geber, 2010). Larger population sizes, and substantially higher reproductive, viability and growth-rate fitness in upland than bottomland forests, in conjunction with the historically greater area of upland forests, suggest that the vast majority of successful reproduction and establishment occurs in upland sites. Additionally, we detected significantly asymmetrical gene flow via pollen from upland populations into bottomland populations (Anderson & Geber, 2010). These patterns of highly disparate fitness and unbalanced gene flow are consistent with strong demographic source–sink dynamics, with bottomland populations representing a relative sink.

The objective of the current study was to determine whether V. elliottii exhibits fixed phenotypic traits that enhance fitness in upland habitats, as predicted. Alternatively, phenotypic plasticity could be maintained despite demographic source–sink dynamics. Plasticity can either be favored by selection, or be a passive response to environmental variation (e.g. Sultan & Bazzaz, 1993; Dudley & Schmitt, 1996; Dorn et al., 2000; van Kleunen & Fischer, 2005; Caruso et al., 2006; Ghalambor et al., 2007; Kurashige & Callahan, 2007). Passive plasticity can occur when phenotypes are constrained by environmental conditions in one habitat type, in which case plasticity is neutral or even costly; for example, Arabidopsis thaliana had stunted phenotypes in cold temperatures (Kurashige & Callahan, 2007), as did Geranium carolinianum in response to crowding (Bell & Galloway, 2007). Adaptive and passive plasticity have both been documented within species along environmental gradients, as has been shown with light intensity (Sultan & Bazzaz, 1993; Steiniger et al., 2003) and water availability (Caruso et al., 2006). In our system, it is crucial to consider whether plasticity is passive or adaptive: adaptive plasticity would contradict predictions from demographic source–sink dynamic models, whereas passive plasticity would not conflict with this body of theory.

To assess phenotypic evolution in an ecologically relevant context, we conducted a multi-year reciprocal transplant experiment in drought-stressed upland forests and flood-prone bottomland forests that vary in water-table depth, light intensity and edaphic characteristics. In a glasshouse experiment, we isolated the effects of flooding and drought to determine whether trait values varied in response to differential water stress. Flooding deprives roots of oxygen and can result in leaf senescence, necrosis and abscission, a reduction in photosynthesis and stomatal conductance, and ultimately death (e.g. Blokhina et al., 2003; Mielke et al., 2003; Visser et al., 2003). Flood-adapted species have evolved constitutively expressed and inducible traits that...
facilitate gas exchange between above-water biomass and roots, including aerenchyma (porous root and shoot tissue, Evans, 2003), hypertrophied lenticels and adventitious, lateral or superficial roots (Fenster, 1997; Benz et al., 2007). Drought is also a severe stress that can inhibit ecophysiological performance and lead to a reduction in turgor pressure and potential xylem cavitation (Warren et al., 2004). Some species exposed to drought have thick leaves with low specific leaf area (SLA) and increased water-use efficiency (WUE) to optimize photosynthesis per unit of water transpired (e.g. Donovan & Ehleringer, 1994; Wright et al., 2002). Additionally, deep taproots can exploit water at depth in dry soils (e.g. Wildy et al., 2004). By contrast, flood-adapted plants tend to maintain shallow root systems to access oxygen at the water–air interface (Baker et al., 2001). Furthermore, porous roots that enhance fitness under flooding are likely to desiccate under dry conditions (Fenster, 1997). As selection probably favors opposing trait values in upland and bottomland forests, we quantified root and foliar traits that influence flood and drought tolerance.

If *V. elliottii* conforms to predictions of source–sink dynamics, experimental individuals would display canalized flood-intolerant phenotypes, and trait values would be concordant with drought-tolerance. By contrast, if phenotypic plasticity is maintained, individuals would express the phenotype that best matches the environment. Additionally, we would expect plasticity in flood-related traits, notwithstanding the lack of flooding in upland (source) environments. To distinguish between passive and adaptive plasticity, we assessed performance-related traits, such as photosynthesis and stomatal conductance, as well as foliar and root-based traits that are probably under divergent selection.

**Materials and Methods**

**Study system**

We conducted the reciprocal transplant experiment in four sites within Beidler Forest (33°12′N, 080°18′W), a National Audubon reserve in the Four Holes Swamp watershed of South Carolina. Beidler Forest has c. 6475 hectares of wetland forests with conservation easements in upland forests (N. Brunswig, pers. comm.). We sampled additional populations in the Pee Dee and Santee watersheds, giving a total of 15 bottomland and 17 upland populations (coordinates of the populations are available in the Supporting Information Table S1 in Anderson & Geber, 2010). We provide photographs of adult *V. elliottii* Chapm. individuals in bottomland and upland forests of Beidler Forest in Fig. S1.

Bottomland and upland forests differ substantially in abiotic conditions and floral communities. Floodplain forests are dynamic systems that experience an annual flood/drought cycle which is not present in upland forests (Burke et al., 1999). The poorly drained clay soils of bottomland forests flood gradually over several days as a result of upstream precipitation; these floods are highly variable in duration, frequency and depth (N. Brunswig, M. Dawson, unpublished data, 1977–present). Flooding events vary inter-annually in the southeastern USA (Burke et al., 1999). At our primary field site (Beidler forest), floods range in duration from 3 to 139 d (mean ± SD: 43.6 ± 36 d) and in frequency from one to seven flood events per growing season (N. Brunswig, M. Dawson, unpublished data, 1977–present). Because *V. elliottii* is a woody perennial, individuals in bottomland forests probably experience multiple years with extensive growing-season floods over their life spans. In this system, gradually increasing soil moisture probably serves as a reliable cue for the induction of flood-related traits in phenotypically plastic plants. In contrast to floodplain forests, drought-stress may be pronounced in upland forests as a result of coarse, sandy soils (Megonigal et al., 1997; Burke et al., 1999). Whereas the water table was very close to the soil surface in bottomland forests (0.24 ± 0.1 m, *n* = 4 sites) in a spring with no flooding (2008), the water table was > 1.34 m deep at four upland sites sampled at the same time (J. T. Anderson, unpublished data). Additionally, there is almost complete turnover in plant species composition from upland to bottomland forests (Porcher, 1981), upland forests have significantly greater light penetration into the subcanopy (*n* = 4 upland sites and *n* = 4 bottomland sites; methods and results detailed in Table S1), lower soil moisture and bulk density (*n* = 7 upland sites and *n* = 5 bottomland sites; Table S2) and less fertile soils than floodplain forests (*n* = 4 upland sites and *n* = 4 bottomland sites; Table S3). Historically, upland forests occupied a larger proportion of the landscape than bottomland forests; however, wetland habitats may now predominate because of the destruction of upland habitats (Phillips, 1994; see also discussion in Anderson & Geber, 2010). Species that span subtle elevational gradients in this region must cope with temporal fluctuations in water-table depth in the floodplain, and persist under high levels of spatial variation in environmental conditions.

**Experimental design**

Details of the reciprocal transplant and glasshouse experiments are provided in Anderson & Geber (2010). Briefly, we collected cuttings and seeds from reproductive adults in 2005 and 2006. Cuttings were treated with rooting hormone (Rhizopon AA #3, 0.8% IBA; Rhizopon bv, Hazerswoude, Holland) and grown under automated misting systems until the roots became established. Seeds were germinated in the laboratory. To minimize potential maternal or environmental effects, seedlings and rooted cuttings were grown under benign glasshouse conditions for 6–8 months before experimental manipulations were
carried out. At the start of the experiment, plant size did not vary as a function of habitat of origin \((P > 0.12)\). In contrast to cuttings made from adults, seedlings represented novel combinations of genes not yet exposed to flood/drought cycles in nature. Including both cuttings and seedlings allowed us to detect whether phenotypic expression and/or plasticity altered through ontogeny. In the spring of 2005 and 2006, we transplanted cuttings (both years) and seedlings (2006 only) into two upland and two bottomland transplant sites at Beidler forest \((n = 1685\) cuttings from 399 genotypes and 10 upland and 10 bottomland populations in 2005; \(n = 548\) cuttings from 106 genotypes and 11 bottomland and 10 upland populations and \(n = 814\) seedlings from 81 families and 7 bottomland and 9 upland populations in 2006). We planted two to three cuttings/seedlings per parental adult (genotype/family) in both habitats and have monitored them since outplanting. The bottomland transplant sites flooded in 2005, shortly after transplanting, but did not flood during the 2006–2008 growing seasons, which is unusual for this habitat. In November 2006, we established a glasshouse study at Cornell University to assess the effects of prolonged flooding and drought on the phenotypic traits of \(V.\) elliottii \((n = 271\) seedlings from 87 families of 7 bottomland and 9 upland populations; \(n = 458\) cuttings from 133 genotypes of 12 bottomland and 13 upland populations; Anderson & Geber, 2010). We assigned an average of two individuals from each genotype/family to different blocks in each treatment \((n = 27\) blocks/treatment; range of one to six individuals/family/treatment). We imposed experimental treatments gradually, over 1 month, to provide adequate time for plants to sense and respond to changing conditions. After the final treatment (27 November 2006), we maintained water in the flooded treatment 5 cm above the soil and watered plants in the drought treatment once per week (weekly average volumetric water content: \(c. 8\% \text{ ml water ml}^{-1} \text{(soil + water)}\)) until the end of the experiment (May 2007). The treatments are relevant to stress encountered in the field during extreme years, and treatment levels were based on long-term water-level data from Beidler forest, our soil water measurements in the field, as well as a pilot experiment (further details are provided in Anderson & Geber, 2010). The objective of this study was to expose \(V.\) elliottii individuals to extreme conditions, which they are likely to experience during their lifetimes. We did not include a well-watered control treatment because it would probably not have been relevant to the conditions experienced in the field. Thus, our glasshouse experiment allowed us to detect plasticity in traits, but we could not determine whether this plasticity was caused by phenotypic changes only in one treatment, or in both treatments.

Below, we detail the phenotypic measurements (see Table S4 for an overview) and statistical analyses for both experiments.

Data analyses

All analyses were conducted in SAS (version 9.2; SAS Institute, Cary, NC, USA) with PROC MIXED. We tested the effects, on phenotypic traits, of habitat of origin, transplant environment (field) or treatment (glasshouse), life-history stage and interaction terms. We included random effects for genotype/family nested within population and source population, as well as site nested within transplant environment, in the analyses of the field experiment, and block nested within treatment in the analyses of the glasshouse experiment. Models with source population nested within habitat of origin produce very similar results, but lack denominator degrees of freedom necessary to test the main effect of habitat of origin (results not shown). We computed the significance of random effects using a likelihood ratio test by comparing the \(-2 \times \log \text{likelihoods for models, with and without each random effect, using a one-tailed chi-square test with one degree of freedom (Littell et al., 1996). We} analyzed the two transplant years of the field experiment separately for SLA, but jointly for stable carbon \((C)\) isotopes, in which case, the year of measurement and the year of planting were included in the model of isotopic data. When phenotypic traits were measured multiple times, we conducted repeated-measures ANOVAs. Additionally, when phenotypic traits could be correlated, we ran multivariate analysis of variance (MANOVA) and proceeded to univariate ANOVAs only if the MANOVA produced significant results. Residuals were assessed for normality and homoskedasticity. Significant effects of treatment or transplant environment indicate phenotypic plasticity.

Reciprocal transplant experiment

To assess foliar traits, we harvested green sun and shade leaves from cuttings and seedlings in the reciprocal transplant experiment (mean ± SD: 9 ± 5 leaves per plant) at the end of two growing seasons (during October 2006 and October 2007). To quantify SLA (i.e. leaf area per unit biomass, expressed as cm² g⁻¹), we measured the area of fresh leaves using a leaf area meter (LI-3100; Li–Cor, Lincoln, NE, USA) in 2006; by contrast, in 2007, we quantified the leaf area using digital photography. We photographed fresh leaves on a white sheet of standard-sized paper \((21.59 \times 27.94\text{ cm}²)\) and used Adobe Photoshop to convert the images to black and white and to calculate the area of black (leaf) pixels. These two methods produced nearly identical results \((\beta = 1.01 \pm 0.004, F_{1,28} = 70090, P < 0.0001, n = 29)\). We used repeated-measures ANOVA to analyze the first-year and second-year SLA data from the 2005 transplants (repeated statement for year with autoregressive correlation structure). Leaves were collected from the 2006 transplants only in the fall of 2007; a mixed-model ANOVA assessed the effects of predictors on SLA for these individuals.
We used a subset of these leaves to determine foliar nitrogen (N) content (%N, which is correlated with photosynthetic capacity, Evans, 1989) and stable C isotope ratios ($\delta^{13}$C), which can reflect WUE and the ecophysiology of leaves (e.g., Farquhar et al., 1989). As the leaves were collected in October, $\delta^{13}$C represents an integration of C fixed during the entire growing season (Farquhar et al., 1989). In both 2006 and 2007, we pooled leaves of cuttings from each source population planted into both transplant environments ($n = 17$ source populations in 2006 and 29 populations in 2007, replicated in both upland and bottomland sites), and in 2007 we created additional upland and bottomland pools for each seedling family. The leaves of each pooled sample were dried at 50°C and ground to a fine powder. Isotopic composition was determined by mass spectrometry in the Cornell Stable Isotope Laboratory and expressed relative to the Vienna Pee Dee Belemnite standard. Because a MANOVA was significant (Table S5), we present results from the univariate ANOVAs. We included a repeated effect to account for multiple observations on the same population.

Glasshouse experiment

In May 2007, at the end of the experiment, we harvested leaves, stems and belowground biomass of all living plants. We calculated SLA from a subset of leaves produced during the experiment (mean ± SE: 13.7 ± 0.4 leaves per plant) using digital images imported into Adobe Photoshop. Aboveground biomass was dried at 50°C for 4 d and then weighed. To assess root architecture, we divided the roots into three sections: top 1 cm of soil; 1.1–5 cm deep; and > 5 cm deep. We washed the soil from these samples, then dried and weighed the roots. A common response to flooding is the production of roots at the air–water interface (e.g., Fenster, 1997), but shallow roots could be more prone to desiccation under drought conditions; thus, we predicted that the proportion of roots in the top 1 cm of the soil would be significantly greater for flooded plants than for drought-stressed plants. Additionally, we expected drought-stressed plants to allocate more resources to root production (resulting in a higher root: shoot ratio) than flooded plants. A MANOVA assessed the effects of predictors and their interactions on SLA, root: shoot ratio (natural log transformed), and root architecture (proportion of roots in the top 1 cm of soil). The MANOVA was highly significant (Table S6), so we conducted univariate ANOVAs for each response variable.

Root porosity and tissue density We used a subset of seedlings and cuttings to quantify root porosity and root-tissue density ($n = 26$ seedlings, 85 cuttings; 51 of which were from the drought treatment and 60 from the flooded treatment). Root porosity reflects the extent of gas-filled tissue (aerenchyma), which can be produced either constitutively, or induced by flooding (Evans, 2003). We selected fine roots from the top 5 cm of the soil and determined root porosity using the microbalance method of Visser & Bögemann (2003). After measuring porosity, we dried the samples and calculated the ratio of dry to fresh root weight to estimate root-tissue density (e.g., Zobel et al., 2006), which should be lower under flooded conditions than under drought conditions. A MANOVA with root porosity and root-tissue density as response variables detected a significant interaction between treatment and response ($F_{1,91} = 13.05$, $P = 0.0005$); therefore, we ran univariate mixed-model ANOVAs. The random effects of family nested within population, family by treatment, and block nested within treatment were evaluated and were removed from the models because of nonsignificance, which probably resulted from small sample sizes for these measurements (genotypes and families were only represented by one individual from each treatment). To conserve statistical power, the analysis of root-tissue density did not include the three-way interaction, or the interaction between treatment and life-history stage. An arcsine (square root) transformation improved the slightly heteroskedastic root-porosity residuals, but did not alter the statistical results.

Leaf physiology Both flooding and drought can reduce photosynthesis through diminished stomatal conductance (Mielke et al., 2003; Li et al., 2007) and bottomland and upland genotypes could exhibit divergent physiological responses to these stresses. We used an infrared gas analysis system (LiCor 6400; Li–Cor) to quantify photosynthesis, stomatal conductance and instantaneous WUE (= photosynthesis + transpiration, Donovan & Ehleringer, 1994). Instantaneous WUE was positively correlated with photosynthesis/stomatal conductance ($A + g_s$; $F_{1,230} = 599.2$, $P < 0.0001$) and inversely correlated with internal CO₂ concentration ($c_i$; $F_{1,230} = 412.1$, $P < 0.0001$); therefore, we did not consider these alternative metrics of WUE.

We selected two to three individuals per genotype of cuttings from both treatments for these measurements ($n = 123$ total plants from 44 genotypes; bottomlands, $n = 22$ genotypes from 8 populations; uplands, $n = 22$ genotypes from 9 populations). Seedlings were not included in this aspect of the study. Before starting the experiment, we gathered baseline data (Table S7). We then made two sets of measurements: after experimental conditions had been imposed for 1 month (December 2006); and near the end of the experiment (April 2007). Measurements were made between 09:00 and 17:00 h at 1500 µmol m⁻² s⁻¹ photosynthetically active radiation (PAR), 400 µmol mol⁻¹ CO₂ and 56.0 ± 0.66% (mean ± SE) relative humidity. The average of more than six measurements was calculated for each leaf, and at least two to three leaves were used per plant. For each set of measurements, we randomly sampled
plants over 3–4 d, draining flooded individuals and providing a small amount of water to plants in the drought treatment.

We conducted repeated-measures ANOVAs separately for photosynthesis, stomatal conductance and WUE. Individuals that died over the course of the experiment were included in the analysis, but obviously no values were available after their death. One common response to flooding was leaf senescence; individuals were given values of zero when they had no leaves. Several individuals exhibited negative conductance values, which are biologically meaningless and can be reported when the stomata are closed and conductance is very low; we adjusted negative values to zero.

Natural log transformation improved the residuals for conductance (+0.01 because of zero values) and photosynthesis (+2 because of negative and zero values).

At the end of the experiment, we used stable C isotopes to assess the physiological responses of plants to drought and flooding. We collected leaves that had been produced over the course of the experiment and pooled the leaves for each population and treatment (n = 66 pools). Samples were processed as described for the reciprocal transplant experiment.

Results

Reciprocal transplant experiment

V. elliottii showed consistent plasticity for SLA; across years, SLA was significantly higher in bottomland forests than in upland forests (Table 1, Fig. 1a). For the 2006 transplants, cuttings had a significantly lower SLA than seedlings (significant effect of life-history stage, Table 1).

Stable C isotope ratios varied by transplant environment ($F_{1,27} = 127, P < 0.0001$, Fig. 1b), the year of sampling ($F_{1,16} = 5.2, P = 0.036$) and the year of planting ($F_{1,11} = 6.2, P = 0.03$). Similarly, %N varied by transplant environment ($F_{1,27} = 123.8, P < 0.0001$, Fig. 1c). No other main effects or interactions were significant. The foliar C : N was significantly greater in upland forests than in bottomland forests ($F_{1,27} = 274.2, P < 0.0001$), concurrent with differences in soil chemistry (Table S3).

Glasshouse experiment

Univariate analyses revealed significant variation in root and foliar traits in the glasshouse experiment (see Table S8 for full models). Flooded plants had significantly greater allocation to shallow roots ($F_{1,52} = 63.8, P < 0.0001$, Fig. 2a) and significantly lower root : shoot ratios than drought-stressed plants ($F_{1,52} = 108.7, P < 0.0001$, Fig. 2b). Seedlings had a larger proportion of shallow roots than cuttings ($F_{1,202} = 88.0, P < 0.0001$); this difference was accentuated in the flooded treatment (life history × treatment: $F_{1,202} = 7.2, P = 0.0081$). Specific leaf area was significantly lower in flooded treatments than in drought treatments ($F_{1,52} = 40.9, P < 0.0001$, Fig. 2c) and seedlings had a higher SLA than cuttings (LSMEANS ± SE: seedlings: 156.1 ± 3.3; cuttings: 136.8 ± 2.5 cm$^2$ g$^{-1}$; $F_{1,181} = 22.5, P < 0.0001$). Finally, concordant with predictions, root porosity was significantly greater in flooded plants than in drought-stressed plants ($F_{1,103} = 6.5, P = 0.012$, Fig. 2d), whereas root-tissue density showed the opposite pattern ($F_{1,95} = 4.8, P = 0.03$, Fig. 2e).

Ecophysiological traits varied by month of measurement, treatment and the month by treatment interaction (Fig. 3 and Table 2). Photosynthesis and stomatal conductance continuously declined through time in the flooded treatment. In the drought treatment, however, these physiological

Table 1 Results for foliar traits measured in a reciprocal transplant experiment: repeated-measures ANOVA on specific leaf area (SLA) of leaves collected in October 2006 and 2007 from the 2005 transplants; and mixed-model ANOVA of SLA of leaves collected in October 2007 from the 2006 transplants

<table>
<thead>
<tr>
<th>Source</th>
<th>Repeated-measures SLA (2005 transplants)</th>
<th>SLA (2006 transplants)</th>
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<tr>
<td></td>
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<td>F or $\chi^2$</td>
</tr>
<tr>
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</tr>
<tr>
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<td>L × H</td>
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<tr>
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<tr>
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<td>Population</td>
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</table>

The significance of random effects (family nested within source population, source population, and site nested within environment) was assessed using likelihood ratio tests ($\chi^2$, degrees of freedom = 1). Fixed effects were assessed via F-tests. Significant P-values are in bold. NA (Not applicable) is printed for effects not measured for the 2005 transplants.
parameters began to recover by the end of the experiment (late April), which is evident in the significant time by treatment interaction for photosynthesis and conductance. Water use efficiency decreased through time in both treatments. There was significant population-level variation for photosynthesis and conductance. Carbon isotope ratios (δ13C) did not differ between the flooded (mean ± SE: −27.6 ± 0.27) and drought (−28.1 ± 0.25; \( F_{1,34} = 2.08, P = 0.16 \)) treatments, and no other effects or interactions were significant.

Discussion

*V. elliottii* families exhibited phenotypic plasticity for almost all of the traits measured in both experiments. We found no evidence, however, that trait values or reaction norms varied with habitat of origin. Furthermore, seedlings and cuttings did not differ substantially in phenotypic plasticity, as shown by nonsignificant life history by treatment or environment interactions for all traits, except for root architecture in the glasshouse experiment. Previous analyses revealed virtually no neutral population divergence in this heterogeneous landscape (Anderson & Geber, 2010), which accords well with the high degree of plasticity. However, fitness was significantly greater in upland sites than in bottomland sites, and under drought-stress relative to flooding in the glasshouse (Anderson & Geber, 2010). Similarly, photosynthetic and stomatal conductance rebounded in the drought treatment at the end of the glasshouse experiment, but continued to decline in the flooded treatment, indicating that *V. elliottii* populations are better adapted to long-term drought than flooding (Fig. 3). This result substantiates previous conclusions of demographic source–sink dynamics in this species (Anderson & Geber, 2010). According to source–sink theory (Holt & Gaines, 1992; Kawecki, 1995), *V. elliottii* should display traits that enhance fitness in upland forests at the expense of fitness in the stressful bottomland. The phenotypic plasticity documented in this study contradicts this theoretical expectation (Dias, 1996).

Adaptive plasticity occurs when genotypes optimize fitness across environments by altering trait values in response to specific cues to facilitate phenotype–environment matching (e.g. Dudley & Schmitt, 1996). Alternatively, passive plasticity in performance-related traits can be a response to limited resources or stressful conditions in one habitat (e.g. Sultan & Bazzaz, 1993; Dorn et al., 2000; van Kleunen & Fischer, 2005; Caruso et al., 2006; Ghalambor et al., 2007;
In our system, *V. elliottii* probably displays both adaptive and passive plasticity. For example, plasticity in photosynthesis and stomatal conductance was probably maladaptive, because it indicated lower performance under flooded conditions than drought stress at the end of the glasshouse experiment. However, the direction of the plasticity we documented was generally concordant with predictions, for example, higher root porosity, greater allocation to shallow roots and lower root : shoot ratios under flooded conditions, and thinner leaves with greater N content in shady, nutrient-rich bottomland forests. These trait values enhance performance under similar environmental conditions in other species (e.g. Fenster, 1997; Baker *et al.*, 2001; Wright *et al.*, 2002; Evans, 2003; Wildy *et al.*, 2004). Genotypic selection analyses indicate that plasticity in the root : shoot ratio and SLA was adaptive in response to water stress in the glasshouse and that genotypes with greater plasticity in rooting depth performed better under drought (Anderson, 2009; J.T. Anderson, unpublished). These analyses uncovered selection against plasticity in SLA in the field, but limited costs to plasticity in other traits (Anderson, 2009; J.T. Anderson, unpublished).

There are several explanations for the existence of phenotypic plasticity in a system where fitness varies by habitat. For one, natural selection could favor plasticity in the source habitat in response to within-habitat spatial variation. In our system, plasticity could be advantageous in upland populations of *V. elliottii* as a result of subtle spatial variation in light intensity or edaphic conditions. In this case, we would expect limited plasticity in traits related to flooding tolerance because upland systems do not experience floods. Alternatively, habitat-based differences in fitness in our system might not reflect long-term source–sink dynamics (e.g. Holt, 1997), but rather incipient expansion in niche breadth. Phenotypic plasticity can enhance range and niche expansion (e.g. Schlichting & Smith, 2002), but fitness could initially be limited in the novel habitat. Phenotypically plastic *V. elliottii* genotypes could spread faster in both habitat types, even if fitness is lower overall in bottomland forests. Finally, it is possible that phenotypic plasticity is a phylogenetic legacy, especially if the costs of plasticity are low and/or there is minimal genetic variation for plasticity. Examination of the traits measured in the glasshouse and in the field can address these issues.

**Foliar traits**

We found substantial plasticity in SLA, N content and stable C isotope ratios in the field, even though flooding did not occur during the years when traits were measured; however, there was limited to no plasticity in the glasshouse. Water stress was the only factor that varied in the glasshouse, whereas a suite of conditions differed between habitats in the field, including water-table depth, light intensity and soil nutrient content (Tables S1–S3). In other wetland species, flooding and complete submersion result in thinner leaves (i.e., higher SLA) (e.g. Mommer *et al.*, 2006; Huber *et al.*, 2009). Our glasshouse results, by contrast, suggest that flooding and drought induce similar foliar traits: thick leaves (resulting in a low SLA) and low internal leaf CO₂ concentration (resulting in high C isotope ratios). Taken together, our glasshouse and field results suggest that habitat-based differences in light and edaphic factors could be responsible for foliar plasticity in nature. Consistent with the results from other studies (e.g. Meziane & Shipley, 1999; Steinger *et al.*, 2003; Galloway & Ettlerson, 2009), our transplants had significantly thinner leaves under shady, nutrient-rich conditions (bottomland forests) than sunny,
nutrient-poor conditions (upland forests). Additionally, enhanced light availability in the glasshouse could have produced thicker leaves in both treatments, reducing the SLA to below the values encountered in the field. Repeated-measures ANOVA on SLA values of experimental individuals transplanted in 2005 showed a significant effect of measurement year (Table 1), indicating temporal plasticity that might accord with yearly fluctuations in abiotic conditions. This flexibility in leaf anatomy probably improves plant performance in temporally variable habitats.

Owing to the close relationship among SLA, foliar N content and photosynthesis (Shipley et al., 2006), it is not surprising that we found a high degree of phenotypic plasticity in ecophysiology in the field (Fig. 1b,c). Discrimination against the heavier $^{13}$C isotope relative to the more abundant $^{12}$C isotope (i.e. fractionation) occurs during CO$_2$ diffusion through the stomata and boundary layer, internal CO$_2$ transfer through the mesophyll, carboxylation and photorespiration (Seibt et al., 2008). The lower C isotope ratios of bottomland transplants relative to upland transplants reflect a higher ratio of internal leaf CO$_2$ concentration to atmospheric CO$_2$ concentration ($\delta$; $c_a$), which can be caused by increased stomatal conductance or decreased carboxylation capacity (Farquhar et al., 1989). The higher elemental N (%N) of bottomland transplants is indicative of increased photosynthetic capacity because foliar N is allocated primarily to proteins involved in photosynthesis (e.g. Evans, 1989). Therefore, the $\delta^{13}$C values from the reciprocal transplant experiment probably signify higher stomatal conductance in bottomland transplants relative to upland transplants (Farquhar et al., 1989; Dawson et al., 2002). We found substantially higher $\delta^{13}$C values in both glasshouse treatments than in the field. Thus, glasshouse plants in both treatments had lower internal leaf CO$_2$ concentration, presumably lower stomatal conductance and greater WUE than in the field. All plants in the glasshouse were exposed to the same light conditions, whereas light conditions differ significantly between upland forests and bottomland forests in nature (Table S1). Therefore, the significant differences found between bottomland and upland transplants in stable C isotope ratios could be a response to habitat-based variation in light.

Flood tolerance

In the glasshouse, flooding did not induce the enlargement of lenticels or the production of adventitious roots, even though these traits facilitate gas exchange with flooded roots in other wetland plants and are often produced rapidly after flooding begins (e.g. Fenster, 1997; Mielke et al., 2003). Nevertheless, V. elliottii did express flood-tolerant traits, contrary to source–sink theory predictions. Flooded individuals had a higher proportion of their roots in the top 1 cm of the soil, and proliferation of shallow roots at the air–water interface can enhance plant performance under flooded conditions (Fenster, 1997). Additionally, flooding altered the root : shoot ratios, root porosity and root-tissue density in predicted directions (e.g. Visser & Bögemann, 2003; Mommer et al., 2006). It seems unlikely that plasticity in these traits would be favorable within drought-prone upland habitats because porous roots would probably desiccate under drought (Fenster, 1997), and shallow roots would be disadvantageous in water acquisition (e.g. Wildy et al., 2004).

Examining the influence of phylogeny could illuminate how V. elliottii spans such a broad gradient of environmental conditions. The genus Vaccinium occupies a wide range of habitats, with broadly varying moisture regimes, and many
species occur in both wetland and upland systems (US Fish and Wildlife Service: http://www.fws.gov/nwi/bha/downloads/1996/national.pdf). Although few ecological studies have been conducted on the flood tolerance of Vaccinium species, the horticultural literature reveals that the closely related Vaccinium corymbosum (Bruederle & Vorsa, 1994) produces aerenchyma (porous root tissue) in response to flooding (Abbott & Gough, 1987). Additionally, cranberries (Vaccinium macrocarpon), native to North American bogs, are cultivated in flooded conditions (Sandler et al., 2007) and are probably highly flood tolerant.

Conclusions

Phenotypic plasticity in certain traits, such as SLA and stable C isotope ratios, could be an adaptation to light or edaphic conditions that vary in upland forests. However, temporal and spatial variation in upland forests cannot account for plasticity in flood-related traits such as root porosity, root-tissue density, root : shoot ratio and rooting architecture because the well-drained sandy soils of upland forests do not flood. Plasticity in flooding tolerance could be maintained if bottomland habitats occupied a greater proportion of the landscape than higher-quality upland habitats. Historical records, however, indicate that upland forests probably predominated in this system before large-scale human alteration of the landscape (Anderson & Geber, 2010). Instead, plasticity in flood-related traits could be the result of phylogenetic history, perhaps occurring in concert with minimal costs of plasticity, or limited genetic variance in plasticity. Despite the unresolved phylogeny of Vaccinium, we know V. elliottii to have recent common ancestors with wetland specialists, such as V. corymbosum (Bruederle & Vorsa, 1994). Additionally, plasticity for water stress-related traits may have aided V. elliottii in range-expansions and help explain the current distribution pattern. We propose that phenotypic plasticity in foliar and root traits allows V. elliottii individuals to colonize and persist in stressful bottomland forests; however, source–sink dynamics probably decrease the capacity of this species to evolve additional flood-tolerant traits.

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**Supporting Information**

Additional supporting information may be found in the online version of this article.

**Fig. S1** Photographs of adult *Vaccinium elliottii* in upland and bottomland habitats.

**Table S1** Habitat-based differences in light intensity

**Table S2** Habitat-based differences in soil moisture content and soil bulk density

**Table S3** Habitat-based differences in soil fertility and percentage organic matter

**Table S4** Phenotypic traits measured in the field and glasshouse experiments

**Table S5** MANOVA assessing the effects of habitat of origin (H), transplant environment (Env), and life-history stage (L) on carbon isotope ratios ($\delta^{13}$C) and foliar nitrogen content (%N)

**Table S6** MANOVA assessing phenotypic traits of individuals in the glasshouse experiment

**Table S7** Baseline ecophysiological data, recorded in September 2006, before the initiation of the glasshouse experiment

**Table S8** Univariate ANOVA results of phenotypic traits measured in the glasshouse experiment

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