An experimental test of fitness variation across a hydrologic gradient predicts willow and poplar species distributions

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Abstract. Environmental filtering is an important community assembly process influencing species distributions. Contrasting species abundance patterns along environmental gradients are commonly used to provide evidence for environmental filtering. However, the same abundance patterns may result from alternative or concurrent assembly processes. Experimental tests are an important means to decipher whether species fitness varies with environment, in the absence of dispersal constraints and biotic interactions, and to draw conclusions about the importance of environmental filtering in community assembly. We performed an experimental test of environmental filtering in 14 closely related willow and poplar species (family Salicaceae) by transplanting cuttings of each species into 40 common gardens established along a natural hydrologic gradient in the field, where competition was minimized and herbivory was controlled. We analyzed species fitness responses to the hydrologic environment based on cumulative growth and survival over two years using aster fitness models. We also examined variation in nine drought and flooding tolerance traits expected to contribute to performance based on a priori understanding of plant function in relation to water availability and stress. We found substantial evidence that environmental filtering along the hydrologic gradient played a critical role in determining species distributions. Fitness variation of each species in the field experiment was used to model their water table depth optima. These optima predicted 68% of the variation in species realized hydrologic niches based on peak abundance in naturally assembled communities in the surrounding region. Multiple traits associated with water transport efficiency and water stress tolerance were correlated with species hydrologic niches, but they did not necessarily covary with each other. As a consequence, species occupying similar hydrologic niches had different combinations of trait values. Moreover, individual traits were less phylogenetically conserved than species hydrologic niches and integrated water stress tolerance as determined by multiple traits. We conclude that differential fitness among species along the hydrologic gradient was the consequence of multiple traits associated with water transport and water stress tolerance, expressed in different combinations by different species. Varying environmental tolerance, in turn, played a critical role in driving niche segregation among close relatives along the hydrologic gradient.

Key words: aster fitness models; environmental filtering; field experiment; functional traits; hydrologic niche segregation; phylogenetic signal; Salicaceae.

Introduction

Understanding mechanisms of community assembly and the maintenance of biodiversity are central concerns in community ecology. Environmental filtering has long been recognized as an important assembly process driving shifts in species composition (e.g., Whittaker 1960) and functional attributes (Diaz et al. 1998, Cavender-Bares et al. 2004, Cornwell and Ackerly 2009) along environmental gradients. The term "environmental filtering" has been broadly used to refer to the phenomenon in which not all species that arrive in a given location from the regional pool become members of the local community; rather, only those with appropriate characteristics establish and

persist in the habitat, given the environment (Van der Valk 1981, Bazzaz 1991, Woodward and Diament 1991, Weiher and Keddy 1995, reviewed in Kraft et al. 2015a). One challenge in studying environmental filtering is the separation of the effects of multiple community assembly processes on the observed species distribution patterns. Besides abiotic stresses, biotic interactions and dispersal can also act as environmental filters because they can change along environmental gradients and exclude species from unsuitable habitats. For instance, Fine et al. (2004, 2006) found that the damaging effect of herbivory became more severe on species native to nutrient-rich soils when they were transplanted into nutrient-poor soils, restricting these species to their native habitats. Beyond herbivory, interactions among neighboring plants, which can significantly affect plant survival and reproduction, have been shown to shift from competition to facilitation along stress gradients (Callaway et al. 2002). Finally, differential

dispersal (Ehrlén and Eriksson 2000, Tuomisto et al. 2003) and pollination constraints (Sargent and Ackerly 2008, Chalcoff et al. 2012) have also been found to drive contrasting distribution patterns among species across environmental gradients.

To differentiate the effects of these related but different processes on community assembly, Kraft et al. (2015a) recommended that the use of the term "environmental filtering" be restricted to filtering processes caused by abiotic factors (hereafter we will use "environmental filtering" in this narrow sense) and that stringent criteria be used to test for environmental filtering. Observations of shifts in species abundances along environmental gradients are the most common evidence used for inferring environmental filtering (Kraft et al. 2015a). Such observational patterns, when used alone, cannot differentiate shifts in species abundance caused by abiotic factors from alternative community assembly processes that vary along environmental gradients.

Distributions of functional traits within and among communities along environmental gradients have also been used as corroborating evidence supporting conclusions about environmental filtering (Diaz et al. 1998, Weiher et al. 1998, Cavender-Bares et al. 2004, Kraft et al. 2008, Cornwell and Ackerly 2009, Swenson and Enquist 2009). Trait-based approaches can target the mechanisms of environmental filtering more specifically, but only if critical traits that contribute to fitness, given the abiotic environment, can be identified (Violle et al. 2007, Funk et al. 2016, Shipley et al. 2016).

One potential challenge of using trait-based approaches is that multiple traits may be involved in abiotic stress tolerance (Ackerly et al. 2000, Reich et al. 2003, Violle et al. 2007, Cavender-Bares et al. 2009, Losos 2011). In some cases, multiple traits underlying species performance along abiotic gradients covary with each other in a coordinated manner such that the variation in a single trait could well predict differences in stress tolerance among species (e.g., Wright et al. 2004, Chave et al. 2009, Freschet et al. 2010). On the other hand, traits underlying abiotic stress tolerance could also vary among species in a decoupled or antagonistic manner. Consequently, there could be different trait combinations conferring the same level of stress tolerance, and the variation in a single trait might not accurately predict the variation in stress tolerance among species. Two mechanisms could lead to non-coordinated relationships among traits. First, different species could use different strategies to tolerate the same stress factors (e.g., Huner et al. 1993, Schwilk and Ackerly 2001, Chaves et al. 2002, Bailey-Serres and Voesenek 2008). Second, stress tolerance at the organismal level often depends on the performance of multiple organs (Craine et al. 2012); and traits in different organs may not covary with each other in a consistent manner. For instance, although traits associated with the leaf economic spectrum are known to correlate with each other in leaves, stems, and roots separately, there is mixed evidence about whether or not these traits are integrated across different organs (Chave et al. 2009, Reich 2014).

Whether or not traits underlying stress tolerance covary in a coordinated manner with each other also influences patterns of phylogenetic signal in traits as compared to phylogenetic signal in species niches along environmental gradients. If traits underlying species performance along environmental gradients covary in a consistent manner, individual traits should show the same phylogenetic signal as the niches. Alternatively, if variations in traits among species are decoupled from each other, species occupying the same niche could have different trait combinations, causing phylogenetic signals in individual traits to be different from the phylogenetic signal of the niche (Cavender-Bares et al. 2009, Gerhold et al. 2015).

Another challenge of using trait-based approaches to test for environmental filtering is that linking functional traits to fitness in a given environment is not possible without experiments. Experiments designed to test for species fitness responses along environmental gradients, with appropriate control of factors beyond the abiotic environment that influence community assembly, provide more direct evidence for environmental filtering than observational approaches based on species abundance or functional traits (Kraft et al. 2015a). Although field experimental approaches have long been used to examine community assembly processes (e.g., Connell 1961, Paine 1974, Keddy 1981, Bertness and Ellison 1987), in more recent literature, only a few studies have taken an experimental approach to test environmental filtering (but see De Steven 1991, Kobe 1999, Emery et al. 2009, Fraaije et al. 2015).

Here we present an experimental test of environmental filtering combined with functional trait analyses. Our study system was a group of closely related willow and poplar species (family Salicaceae) native to Minnesotan wetlands. Previous studies (Savage and Cavender-Bares 2011, 2012) found Salicaceae species in this region differed in their distributions across a water table depth gradient and in drought tolerance traits. These studies provide circumstantial evidence for environmental filtering, mediated by water stresses, along a hydrologic gradient in the Salicaceae species. These studies also found that species natural distributions across the hydrologic gradient were phylogenetically conserved but individual drought tolerance traits were not. These results could be explained if species occupying similar habitats had different combinations of traits that confer the same level of environmental tolerance. However, the previous studies were not designed to determine how species fitness varied with environment, nor how the constellation of species-level traits varied with their optimal environment determined by fitness.

The first goal of this study was to test for environmental filtering and its importance in community assembly of the Salicaceae species using a field experimental approach designed to examine species fitness variation across environment (Appendix S2: Fig. S1). To address this goal, we transplanted cuttings of 14 species into a series of common gardens established along a natural hydrologic gradient,

where competition was minimized and herbivory was controlled. We modeled the optimal water table depth for each species based on their fitness in the field experiment, then compared the water table depth optima to species distributions across a hydrologic gradient in naturally assembled communities in this region.

The second goal of the study was to examine the relationships among traits underlying species tolerance to environmental stress factors. To address the second goal, we analyzed a suite of functional traits associated with water transport and water stress tolerance. We compared the phylogenetic signal of each trait with the phylogenetic signal of species natural distributions along a water table depth gradient and species integrated stress tolerance (assessed using species fitness variation across the hydrologic gradient). We addressed three specific questions: (1) Do traits of species vary with their hydrologic environment as expected based on a priori understanding of the physiological functions of those traits? (2) Do species occupying similar hydrologic environments have similar combinations of traits? (3) How does phylogenetic signal of individual traits compare to phylogenetic signal of species' natural distributions along the hydrologic gradient and their integrated tolerance to drought and flooding stress?

Materials and Methods

Study site

The study was conducted at Cedar Creek Ecosystem Science Reserve (CCESR) in central Minnesota, USA (45°24′0″ N, 93°12′0″ W). The local climate is continental, with a mean annual average daily temperature of 6.3°C and a mean annual total precipitation of 776 mm based on records from 1963 to 2012 (CCESR weather database, *available online*).[4] The soils are sandy and poor in nutrients (Grigal and Homann 1994). The reserve has a mosaic landscape consisting of numerous patches of prairie, wooded uplands, and wetlands (Appendix S2: Fig. S2). With the exception of several bogs, the majority of wetlands at CCESR share the same regional water table. Due to this feature of hydrology, most wetlands at CCESR have synchronized seasonal variation of water table depth, which peaks in early spring due to snowmelts then gradually declines as the growing season proceeds.

Species distributions across a hydrologic gradient in naturally assembled communities

Fourteen native Salicaceae species, including 13 willows (genus *Salix*) and one poplar (genus *Populus*), naturally occur within CCESR (Appendix S1: Table S1). Savage and Cavender-Bares (2012) found in a field survey that these species had differential distributions along a water table depth gradient. Briefly, they established 50

10 × 30 m plots at randomly chosen locations within CCESR and three preserves nearby (Helen Allison Savanna Scientific and Natural Area, 45°22′48″ N, 93°10′48″ W; Boot Lake Scientific and Natural Area, 45°20′24″ N, 93°7′12″ W; and Carlos Avery Wildlife Management Area, 45°20′42″ N, 93°01′12″ W), and measured the total basal area of the species and monthly water table depth in these plots from 2008 to 2009. Two metrics were calculated based on species abundance and water table depth in the plots, which were mean water table depth averaged across all plots weighted by species abundance in each plot during the wettest month (May, WT_{wet}) and the driest month (August, WT_{dry}) of the growing seasons (Appendix S1: Table S1). WT_{wet} ranged from −102 to 7 cm across species; WT_{dry} ranged from −110 to 0 cm (negative values indicate that the water table was below the ground level; positive values indicate that the water table was above the ground level). Due to the synchronization of water table fluctuation among the wetlands at the study area, the two distribution metrics were highly correlated among species, with a Pearson correlation coefficient of 0.94.

Field experiment

The experiment included 40 common gardens located at 10 different sites at CCESR (Appendix S2: Fig. S2). Each site had a natural wetland, around which four common gardens, separated into two pairs, were built. The two gardens in each pair were located at the two ends of a transitional zone connecting the wetland to its adjacent upland (Appendix S2: Fig. S2). All common gardens had a 17 × 5 m rectangular shape. To minimize the amount of water table depth variation within the gardens, garden locations were chosen such that vegetation within the gardens was mostly homogenous, and the gardens were orientated in a way such that their short sides were parallel to the directions of the upland–wetland gradient.

Twenty-eight cuttings, two from each species, were planted in each garden. The two conspecific individuals were always planted next to each other; and the locations of the conspecific pairs were randomly arranged within the gardens. One advantage of using cuttings instead of seeds was that it allowed us to obtain genetically identical individuals. Whenever enough plant materials were available, we planted four conspecific cuttings propagated from the same maternal plant into the same upland–wetland common garden pair. As a consequence, variation in performance among plants grown in paired upland and wetland gardens should not be caused by differences in their genotypes. We were able to control for genotype in this manner in 87% of the experimental plants. To reduce competition between experimental plants, as well as between experimental plants and naturally occurring vegetation, adjacent cuttings were planted 1.4 m away from each other; and a piece of 1 × 1 m landscape fabric was installed beneath each plant to inhibit

[4] https://www.cedarcreek.umn.edu/research/weather

the growth of native vegetation. To exclude large mammalian herbivores (e.g., deer) the gardens were surrounded by metal fences 2.5 m in height.

The experimental design also included a cage treatment nested within gardens as a part of a separate study on insect herbivory (Appendix S2: Fig. S2). During growing seasons, one individual of each conspecific pair was enclosed in a sham cage, and the other individual was enclosed in a real cage. The cages were made from fine white polyester mesh and had a cuboid shape of 0.5 × 0.5 × 1.2 m. Some cages were extended to 2.4 m tall during the second year to accommodate two fast-growing species (*S. eriocephala* and *S. lucida*). The designs of real and sham cages were the same, except that sham cages had openings on their walls that allowed insects to access the experimental plants.

Experimental plants were propagated from branch cuttings collected from natural populations of the Salicaceae species at CCESR and three preserves nearby (described previously). In 2009 and 2010, 13–31 maternal plants per species were sampled to capture a wide range of genotypic variation within species. Cuttings were soaked in tap water for a few days to root. Rooted plants were then grown in individual pots in a greenhouse room at the University of Minnesota, St. Paul. In fall 2010, these plants were transplanted into common gardens after a several-day-long adjustment period in the field during which they were kept in pots.

We measured survival and basal area of experimental plants at the end of the second growing season. The water table depth in each garden was measured once every other week during the 2012 growing season from a well installed at the center of the garden, using a steel tape measure. The wells were made by attaching a PVC well point (Campbell Manufacturing, Betchelsville, Pennsylvania, USA) to a section of PVC pipe of the same inner diameter (5 cm), and were installed 0.6 m and 1.5 m belowground in wetland and upland gardens, respectively. The well points had a closed bottom and fine slots on their walls that allowed water to move in and out of the wells. Besides drought and flooding, nutrient availability could also vary along hydrologic gradients (Silvertown et al. 2015) and influence plants fitness; therefore we measured nitrogen availability in the common gardens as a covariate of plant fitness. Specifically, plant-available soil nitrogen was measured using ion exchange resin bags at one randomly chosen pair of gardens per site during July–August in 2011. The bags were placed 10 cm below the soil surface, extracted with 2 mol/L KCl, and analyzed for inorganic nitrogen using a colorimetric method (Riggs et al. 2015).

Collection of functional traits data

We measured three traits (lenticel density on a first year branch, lenticel density on the main stem, and stem specific hydraulic conductivity) and compiled data on six other traits from previous studies on a larger set of Salicaceae species (Savage and Cavender-Bares 2012).

The traits were chosen for their known importance for water transport and/or tolerating drought or flooding stress (Appendix S1: Table S2). Below, we describe methods used for collecting the three traits novel to this study and provide references for the published trait data.

Lenticel densities.—Lenticels are the entry points of atmospheric oxygen into plants. Oxygen moves within plants primarily by diffusion (Armstrong and Armstrong 2014); therefore, lenticels on the stem or trunk near the water table are particularly important for flooding tolerance (Armstrong 1968), as oxygen that enters through these lenticels travels less distance to waterlogged tissues. Besides diffusion, in some wetland species, oxygen could also move within plants via convective flows. In these plants, lenticels on young branches may also be relevant for flooding tolerance.

In fall 2014, we measured lenticel densities on the lower part of the main stem and on a first-year branch of each experimental plant. We counted the number of lenticels on a 3 cm long section of the main stem and a randomly selected first year branch. Lenticel densities were calculated as the number of lenticels per square centimeter of surface area of the stem or of the branch, assuming the stem had a cylinder shape and the branch a frustum shape. The sampled main stem sections were right above the water table (if the plant was in standing water) or the highest adventitious roots (if the plant had been flooded earlier in the growing season, which would induce the production of adventitious root) or the ground level.

Stem specific conductivity.—We measured stem specific conductivity based on vessel distributions in five to six two-year-old cuttings from each species. These cuttings were grown in a greenhouse room in Franklinville, New York, USA (42°20′24″ N, 78°27′36″ W) and experienced local climate conditions (Savage and Cavender-Bares 2013). Cross sections were made from one first-year branch per plant. Vessel diameters and sapwood areas were measured by taking micrographs, which were then analyzed using Image J (Schneider et al. 2012). Stem specific conductivity was calculated from vessel diameters following Tyree and Ewers (1991). Average vessel diameters of the species were reported in Savage and Cavender-Bares (2013).

Trait data compiled from previous studies.—Turgor loss point, stomatal pore index, root elongation rate, wood density, maximum photosynthetic rate, and stomatal conductance were collected from natural populations of Salicaceae species in CCESR and were reported in Savage (2010) and Savage and Cavender-Bares (2012). Briefly, all of these traits except for root elongation rate were collected from five to six individuals per species in the driest and the wettest communities within the range of the species' natural distribution (i.e., 10–12 individuals total per species). Sampling individuals across a wide range of the hydrologic gradient allowed us to take into account the plasticity of the traits in response to environmental

variation. Root elongation rate was measured in 40 newly germinated seedlings per species that were grown under favorable conditions in the greenhouse.

Data analysis

All analyses were done using R (R Core Team 2016).

Aster fitness models.—Species fitness responses along the water table depth gradient were analyzed using aster fitness models (Geyer et al. 2007, Shaw et al. 2008) based on survival and basal area growth over two growing seasons. Previous studies in Salicaceae species find that performance during early life history stages plays a critical role in determining individuals' lifetime fitness, because young plants are much more sensitive to drought and flooding than adult plants (Karrenberg et al. 2002, Stokes 2008). Therefore, short-term measurements of survival and growth in young Salicaceae plants should provide a valid proxy for their lifetime fitness across hydrologic gradients.

Different fitness components, such as survival and growth, are usually analyzed separately because they do not follow the same probability distributions. Such separate analyses may yield contrary results, making it difficult to draw conclusions about the overall response of species fitness to environmental gradients. Aster fitness models are designed to analyze multiple fitness components following different statistical distributions jointly. In an aster model, the "response variable" is a vector of random variables; each random variable is a fitness component modeled by its appropriate statistical distribution. As fitness components expressed later in life history depend on those expressed earlier (e.g., a plant will have a biomass of zero if it is dead), the sample size of a fitness component in an aster model is determined by the value of the fitness component expressed previously in the individual's life history. Parameters associated with all fitness components are estimated jointly using the maximum likelihood method. All aster model analyses in this study were performed using the aster package (Geyer et al. 2007).

To test the effect of water table depth in the common gardens and its interaction with species on fitness, we constructed four nested aster models (Appendix S2: Fig. S3) and compared these models using likelihood ratio tests. The four models differed from each other by (1) the shape of fitness response to water table depth, and (2) whether a species by water table depth interaction was included or not. Species fitness was modeled either as a linear or a quadratic function of water table depth. Along the water table depth gradient, intensities of two different stresses (drought and flooding) changed in opposite directions. If species were sensitive to only one type of stress, their fitness would change monotonically across the water table depth gradient, which was modeled by linear functions of water table depth. If species were sensitive to both stresses, their fitness would show hump-shaped responses, which was modeled by quadratic

functions of water table depth. The predictors of fitness in each of the four aster models were (1) a species effect and a linear water table depth effect; (2) a species effect and a quadratic function of water table depth; (3) a species effect, a linear water table depth effect, and the interaction between the two; (4) a species effect, a quadratic function of water table depth, and the interaction between the two. Besides these effects related to species and water table depth, we also included three covariates in all models, which were common garden site, species by cage treatment interaction, and residual of soil nitrogen availability after regressing it on water table depth (we used residuals instead of the original values of soil nitrogen availability because it was correlated with water table depth). To account for the seasonal variation in water table depth, we constructed two sets of aster models using growing season maximum and minimum water table depth in common gardens, respectively (i.e., eight models total).

To infer the importance of environmental filtering for the determination of natural distributions of the Salicaceae species, we performed two analyses comparing species natural distributions and the responses of their fitness in the field experiment. First, we predicted the water table depth at which each species reached its highest fitness based on the best aster model determined by the likelihood ratio tests. We then performed a regression of the water table depth at which species had peak natural abundance against their optimal water table depth predicted by the best aster model. A higher coefficient of determination (r^2) would indicate a stronger effect of environmental filtering on species distributions. Second, we regressed the range of species natural distribution across the hydrologic gradient against the range of water table depth within which they had above-zero fitness predicted by the best aster fitness model. The range of species natural distribution was calculated using the field survey data of Savage and Cavender-Bares (2012), which included species basal area abundance in 50 30 m by 10 m random plots and water table depth in the plots. We calculated the range of natural distribution for each species as the difference between the highest and lowest water table depth among the plots in which the species occurred.

Functional trait analyses.—To determine whether species traits vary with hydrologic environment as expected based on a priori understanding of the physiological functions of the traits, we performed univariate regressions of the water table depth at which the species had peak natural abundance and the optimal water table depth predicted based on the best aster model on each of the traits. These regression analyses tested whether traits were associated with drought or flooding tolerance in the Salicaceae species as we predicted based on the literature (Appendix S1: Table S2). To test whether species that occur in similar hydrologic environment have similar combinations of traits, we tested pair-wise correlations between the traits, and performed a principal component

analysis (PCA) of the traits. Additionally, to examine whether multi-trait phenotype could predict species natural distribution or optimal water table depth better than individual traits, we performed regressions of the water table depth at which species had peak abundance and optimal water table depth on principal components of the trait PCA.

To examine how phylogenetic signal of individual traits compare to phylogenetic signal of species natural distributions along the hydrologic gradient and their integrated tolerance to drought and flooding stress, we analyzed phylogenetic signal using the Blomberg's K statistic (Blomberg et al. 2003) in traits, principal components of the trait PCA, the water table depth at which the species had peak abundance, and water table depth optima predicted by the best aster model. We used a published phylogeny constructed from five chloroplast barcode genes and a nuclear alcohol dehydrogenase gene (Savage and Cavender-Bares 2012) and compared observed K values against two null distributions: a random null distribution calculated by randomly swapping trait values across the tips of the phylogeny 10,000 times and a Brownian Motion null distribution calculated by simulating trait evolution using the sim. char function in the geiger package (Harmon et al. 2008), repeated 10,000 times.

To correct for non-randomness in trait data due to phylogenetic relatedness among the species, we re-ran the above-mentioned regressions using phylogenetically independent contrasts of the traits calculated by the pic function in the ape package (Paradis et al. 2004) if both variables in the regression showed significant phylogenetic signals against random the null distributions and no significant difference from the Brownian motion null distributions. We did not perform phylogenetically corrected PCA (pPCA), because the majority of traits did not show significant phylogenetic signal against random null distributions, thus violating the assumption of pPCA that traits included in pPCA analysis should follow a multivariate Brownian motion model (Revell 2009).

RESULTS

Aster fitness models

In 2012, growing season maximum and minimum water table depth in the common gardens ranged from −120.1 to 25.4 cm and from −162.6 to 11.4 cm respectively (Appendix S2: Fig. S2), covering the full range of the water table depth at which the species had their peak natural abundance across the hydrologic gradient (Appendix S1: Table S1). The growing season maximum and minimum water table depths were strongly correlated with each other (with a Pearson correlation coefficient of 0.92); and the likelihood ratio tests among aster models constructed using the two different water table depth showed similar results. Consequently, we only report the results of the models constructed using growing season

maximum water table depth in the main text and used these results for subsequent analyses. The results of the models constructed using growing season minimum water table depth are reported in the Supporting Information (Appendix S1: Tables S3 and S4, Appendix S2: Fig. S4).

The aster fitness model that included an interaction between species and a quadratic function of water table depth fit the data significantly or marginally significantly better than the other three models (Fig. 1; Appendix S1: Table S5). We further tested the significance of each individual effect in the best-fit model, including the covariates, using likelihood ratio tests. The results (Appendix S1: Table S6) showed that species, water table depth, and site had a significant effect on fitness ($P < 0.01$); species by water table depth had a marginally significant effect on fitness ($P = 0.07$); and residual of soil N availability and the cage treatment by species interaction had no significant effect on fitness ($P = 0.7$ and $P = 1.0$, respectively).

Optimal water table depth of the species predicted by the best aster model ranged from −74.1 to −28.2 cm, and was significantly correlated with the water table depth at which species had natural peak abundance ($r^2 = 0.68$, $P < 0.0001$; Fig. 2c). The range of species natural distributions across the hydrologic gradient showed no significant correlation with the range of water table depth within which the species had above-zero fitness as predicted by the best aster fitness model ($r^2 = 0.08$, $P = 0.34$); 10 out of the 14 species showed a narrower natural distribution range than the ranges of their hydrologic niches as predicted by performance in the field experiment (Appendix S2: Fig. S5).

Functional trait analyses

Seven out of nine traits had a significant correlation with the water table depth at which the species had peak abundance and four had the same signs as expected (Appendix S1: Table S2). Specifically, turgor loss point significantly increased and root elongation rate, stomatal pore index, and stem specific conductivity significantly decreased with the water table depth at which species had peak abundance. By contrast, lenticel density on the young branch, maximum photosynthetic rate, and stomatal conductance significantly decreased with the water table depth at which species had peak abundance, which was the opposite of our prediction. Correlations between individual traits and water table depth optima showed a similar pattern as correlations between traits and the water table depth at which species had peak natural abundance, except that stem specific conductivity had a negative but insignificant correlation with water table depth optima (Appendix S1: Table S2). Three out of four published traits (turgor loss point, root elongation rate, stomatal pore index) showed the same correlations with hydrologic niche as previously found in a larger set of Salicaceae species (Savage and Cavender-Bares 2012).

Although seven traits had a significant correlation with the water table depth at which species had peak abundance, each trait was only correlated with two to four
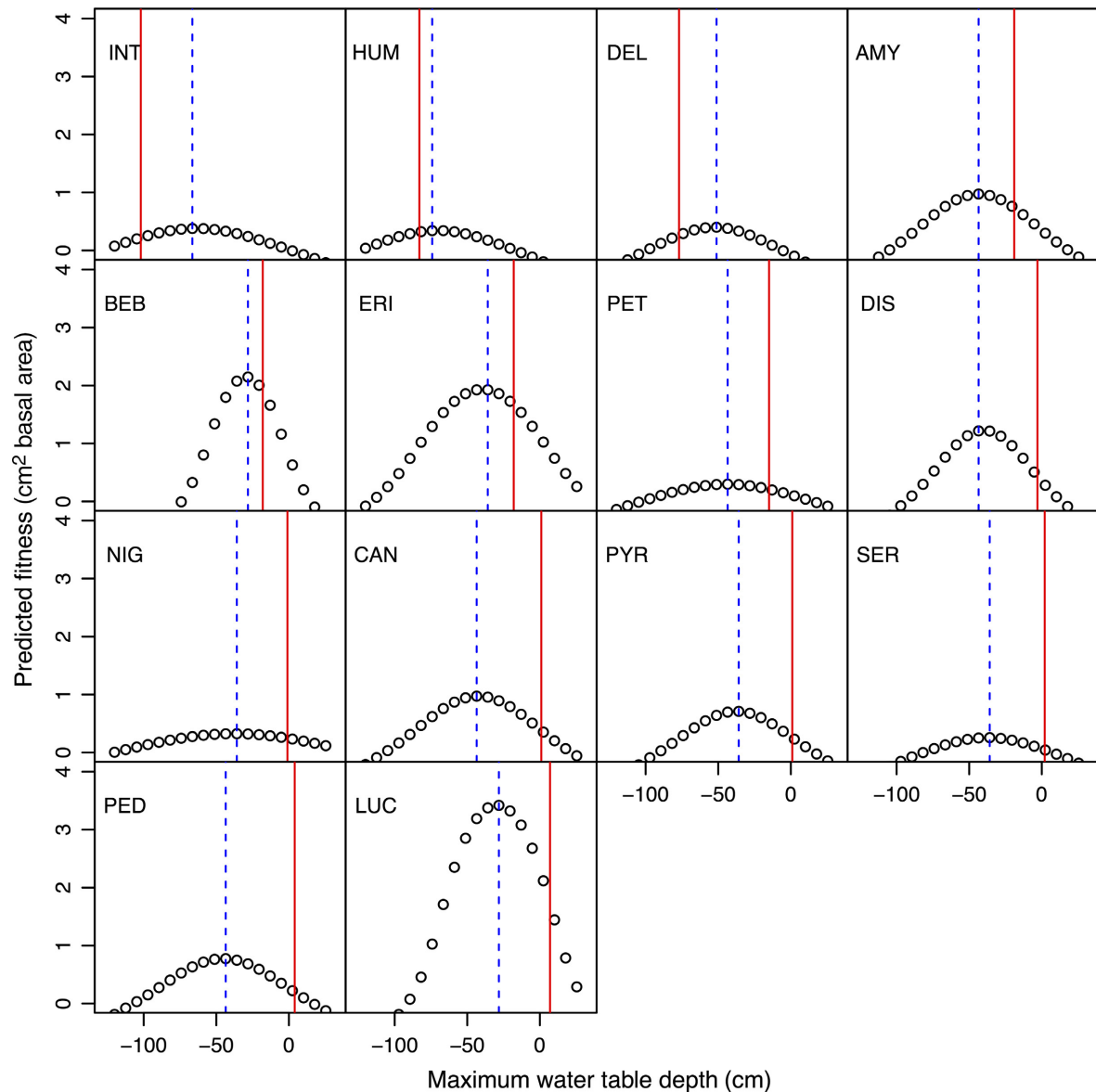
FIG. 1. Species fitness response along the experimental hydrologic gradient predicted by the best aster fitness model. Basal area at the end of the second growing season was used as a surrogate for fitness. A predicted basal area of zero or less indicates that the species could not survive at the given water table depth. Open circles are predicted fitness. Red solid lines indicate the water table depth at which the species had peak natural abundance. Blue dashed lines indicate the water table depth at which the species had the highest predicted fitness. Species names are indicated by the first three letters: AMY, *Salix amygdaloides*; BEB, *S. bebbiana*; CAN, *S. candida*; DEL, *Populus deltoides*; DIS, *S. discolor*; ERI, *S. eriocephala*; HUM, *S. humilis*; INT, *S. interior*; LUC, *S. lucida*; NIG, *S. nigra*; PED, *S. pedicellaris*; PET, *S. petiolaris*; PYR, *S. pyrifolia*; SER, *S. serissima*. [Colour figure can be viewed at wileyonlinelibrary.com]

other traits (Fig. 2a). On a biplot of the first two principal components of the trait PCA (Fig. 2b), traits clustered into two groups: traits in the first group, including stomatal pore index, root elongation rate, wood density, and lenticel density on main stems, were predominantly located in the lower right quadrant, except that turgor loss point was in the upper left quadrant. Traits from the second group, including photosynthetic rate, stomatal conductance, stem specific conductivity, and lenticel

density on young branches, were located in the upper right quadrant. The vectors of two groups of traits pointed in perpendicular directions, indicating that traits within groups were more correlated with each other than with traits in the other group. Three upland species, *S. humilis* (HUM), *S. interior* (INT), and *P. deltoides* (DEL), were scattered in different parts of the plot, indicating they had different trait combinations. The first principal component, which accounted for 48% of the
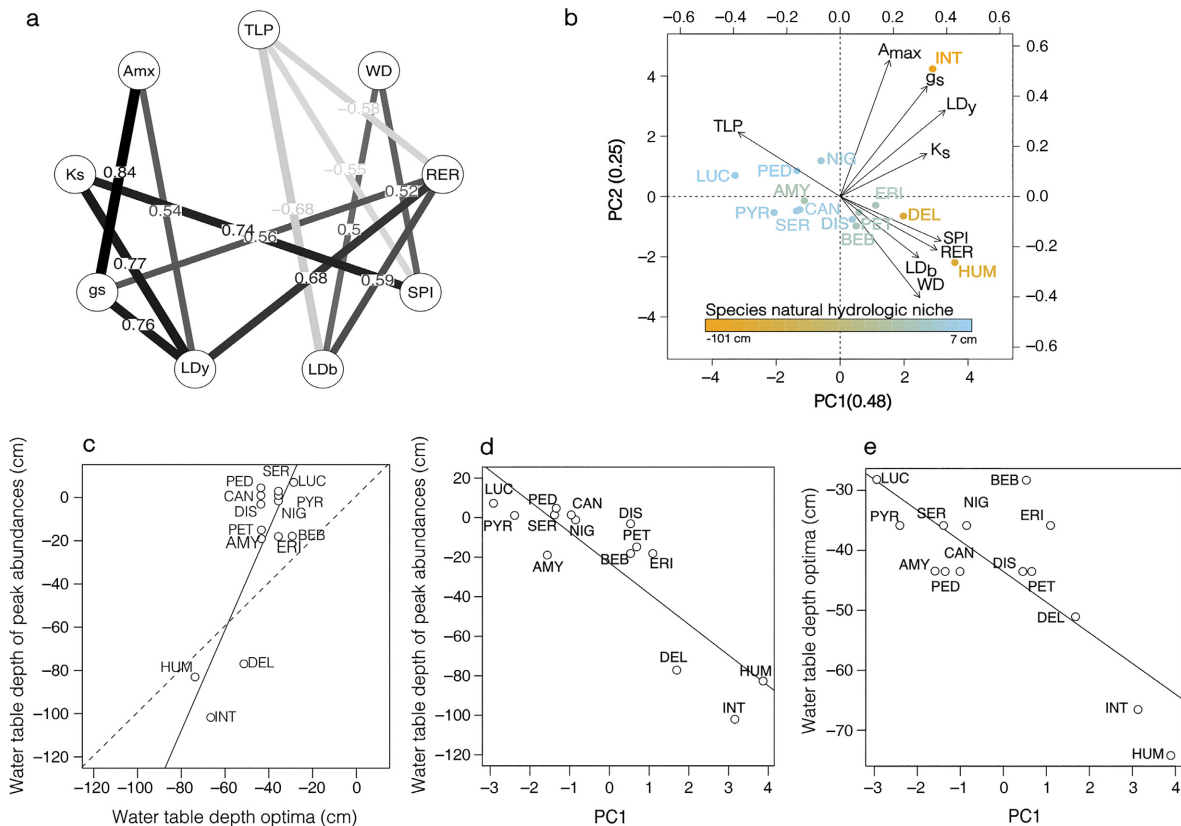
Fig. 2. (a) Correlations among traits. Black and gray lines connecting two traits represent positive and negative correlations, respectively. The intensity of the color indicates the strength of the correlations as determined by absolute values of Pearson correlation coefficients (numbers on lines). Only significant and marginally significant correlations ($P < 0.1$) are shown. Note that not all traits were correlated with each other. Trait names are indicated using the following abbreviations: TLP, turgor loss point; WD, wood density; RER, root elongation rate; SPI, stomatal pore index; LD_b, lenticel density on basal stems; LD_y, lenticel density on young branches; g_s, stomatal conductance; K_s, stem specific hydraulic conductivity; A_{max}, maximum photosynthetic rate. (b) A biplot showing the scores and loadings on the first two principal components from a principal component analysis (PCA) of traits. Species names are indicated by the first three letters; codes are identified in the caption of Fig. 1. The traits were separated into two groups. The first group of traits, including A_{max}, g_s, LD_y, and K_s, are associated with gas exchange and hydraulic conductance. The second group of traits, including TLP, SPI, RER, WD, and LD_b, are mostly morphological traits related to water stresses tolerance. Note that the two groups of traits point in perpendicular directions, indicating traits were more correlated with members within the same group than with traits in the other group. Also note that the three upland species, *S. interior* (INT), *S. humilis* (HUM), and *P. deltoids* (DEL), are located in different places in the biplot, indicating that they had different trait combinations. (c) The water table depth at which species had peak abundance was significantly correlated with the water table optimum predicted by the best aster fitness model ($P < 0.0001$, $r^2 = 0.68$). The solid line is the least square regression line; the dashed line is the 1:1 line. (d) The water table depth at which species had peak abundance was significantly correlated with the first principal component of the trait PCA ($P < 0.0001$, $r^2 = 0.74$). (e) Species water table depth optimum was significantly correlated with the first principal component of the trait PCA ($P = 0.0009$, $r^2 = 0.61$). [Colour figure can be viewed at wileyonlinelibrary.com]

total variance in the data, was negatively correlated with both the water table depth at which species had peak natural abundance and optimal water table depth ($r^2 = 0.74$, $P < 0.0001$ and $r^2 = 0.61$, $P = 0.0009$, respectively; Fig. 2d, e). None of the rest of the principal components was correlated with the water table depth at which species had peak natural abundance or the optimal water table depth. Subsequently, we only tested for phylogenetic signal in the first principal component of the trait PCA.

The observed K values of the water table depth at which species had peak abundance, the predicted water table depth optima, and the first principal component of the trait PCA were significantly higher than the means of

their random null distributions ($K_{obs} = 1.18$, $P = 0.01$; $K_{obs} = 0.75$, $P = 0.04$; $K_{obs} = 0.65$, $P = 0.02$, respectively) and were not significantly different from the means of their Brownian motion null distributions ($P > 0.10$). In contrast, none of the functional traits showed a significantly higher phylogenetic signal than the means of their random null K distributions (Fig. 3). The observed K values of root elongation rate and stomatal pore index were marginally significantly higher than the means of their random null distributions ($K_{obs} = 0.51$, $P = 0.06$ and $K_{obs} = 0.52$, $P = 0.09$, respectively) and not significantly different from the means of their Brownian null distributions. The observed K values of turgor loss point, wood density, photosynthetic rate, and lenticel density on main
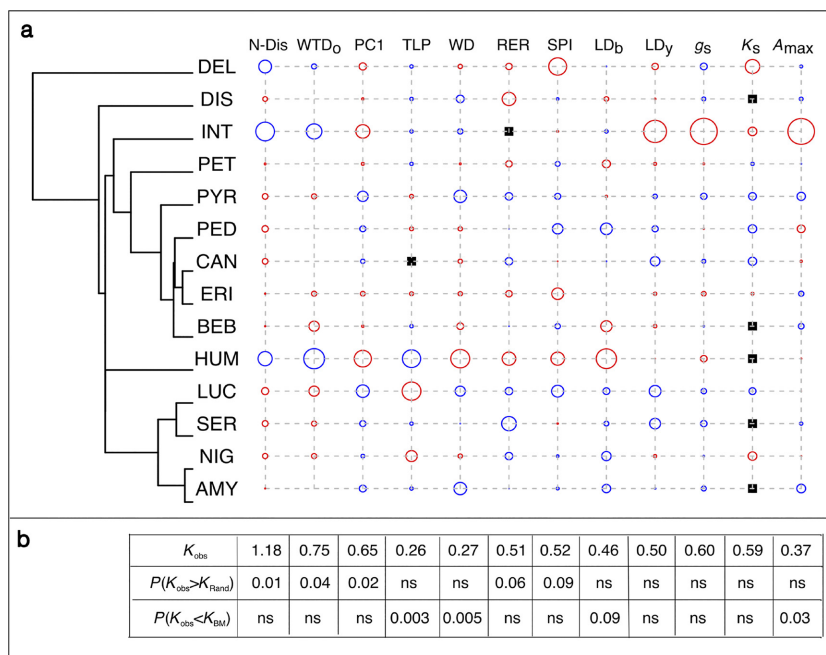
	N-Dis	WTD_O	PC1	TLP	WD	RER	SPI	LD_b	LD_y	g_s	K_s	A_{max}
K_{obs}	1.18	0.75	0.65	0.26	0.27	0.51	0.52	0.46	0.50	0.60	0.59	0.37
$P(K_{obs}>K_{Rand})$	0.01	0.04	0.02	ns	ns	0.06	0.09	ns	ns	ns	ns	ns
$P(K_{obs}<K_{BM})$	ns	ns	ns	0.003	0.005	ns	ns	0.09	ns	ns	ns	0.03

FIG. 3. (a) Distributions of the water table depth at which species had peak abundance (N-Dis), water table depth optimum predicted by the best aster fitness model (WTD_o), the first principal component of the trait PCA (PC1), and functional traits across the Salicaceae phylogeny. Trait names are indicated using abbreviations defined in the caption of Fig. 2. Species names are indicated by the first three letters, defined in the caption of Fig. 1. Circles indicate normalized trait values of the species (trait values were normalized by subtracting the mean of the trait across the species and dividing the remainder by the standard deviation of the trait). Red and blue circles indicate positive and negative values, respectively; diameters of circles indicate absolute values of normalized traits. Black squares indicate missing data. (b) Results of Blomberg's K statistic tests. Observed K values of the traits were compared to random null distributions generated by randomly swapping trait values across species and Brownian motion null distributions; ns, not significant. [Colour figure can be viewed at wileyonlinelibrary.com]

stems were significantly or marginally significantly lower than the means of their Brownian motion null distributions ($K_{obs} = 0.26$, $P = 0.003$; $K_{obs} = 0.27$, $P = 0.005$; $K_{obs} = 0.46$, $P = 0.09$; $K_{obs} = 0.37$, $P = 0.03$) and not significantly different from the means of their random null distributions. The observed K values of lenticel density on young branches, stomatal conductance and stem specific hydraulic conductivity ($K_{obs} = 0.50$, 0.60 and 0.59 respectively) were not significantly different from the means of either their random or Brownian null distributions.

Since the water table depth at which species had peak abundance, the optimal water table depth, and the first principal component of the trait PCA showed significant phylogenetic signal, we performed regression analyses of these traits using phylogenetically independent contrasts (PIC). The results were similar to that of the regular regressions between these traits. The PIC of the water table depth at which species had peak abundance was positively correlated with the PIC of the optimal water table depth predicted by the best aster fitness model ($r^2 = 0.60$, $P = 0.002$); both the PIC of the water table depth at which the species had peak abundance and the optimal water table depth were negatively correlated with the PIC of the first principal component of the trait PCA ($r^2 = 0.52$, $P = 0.005$ and $r^2 = 0.33$, $P = 0.04$, respectively).

DISCUSSION

We found significant fitness variation among 14 Salicaceae species that were experimentally planted along a water table depth gradient in the absence of competition and herbivory. This result provides strong evidence that environmental filtering drove differential distributions among species along an important abiotic gradient. Some of the functional traits linked to water transport efficiency and water stress tolerance varied independently from each other, which we hypothesized allowed species occupying similar habitats to have different trait combinations. As a result, individual traits were less phylogenetically conserved than the water table depth at which species had peak abundance or their integrated stress tolerance determined by multiple traits.

A controlled experiment revealed a critical role of environmental filtering in community assembly

The importance of environmental filtering in the community assembly of the Salicaceae species was shown by the high coefficient of determination (r^2) in the regression of the water table depth at which species had peak natural abundance on the optimal water table depth determined by species fitness in the field experiment (Fig. 2c).

Environmental filtering was caused by both drought and flooding stress, as species fitness showed a quadratic, concave response along the experimental hydrologic gradient in the best aster model (Fig. 1). These results corroborated conclusions of a previous study that found variation in species abundance and functional traits along a hydrologic gradient in naturally occurring Salicaceae populations (Savage and Cavender-Bares 2011, 2012).

Besides environmental filtering, other community assembly processes also influenced species distributions across the hydrologic gradient, as shown by two lines of evidence. First, species fitness optima explained 68%, but not all of, the variation in water table depth at which species had peak abundance. Second, the range of species natural distribution across the hydrologic gradient tended to be narrower than the range of hydrologic niche determined by species performance in the field experiment (Appendix S2: Fig. S5).

A comparison of this study to other experimental work shows that different processes may dominate community assembly of plant species along hydrologic gradients. For instance, Emery et al. (2009) investigated annual plant communities in California vernal pools and found similar results as this study: environmental filtering caused by hydrologic stresses was the dominant process determining species distributions. In contrast, Fraaije et al. (2015) found that among riparian species distributed along a stream hydrologic gradient in the Netherlands, dispersal constraint played a more important role than abiotic stresses. Although these studies found similar patterns of habitat segregation along hydrologic gradients, experimental tests help reveal the underlying differences in community assembly processes.

Integrated stress tolerance of the species was determined by combinations of multiple traits

Several results from our trait analyses showed that not all of the traits associated with water stress tolerance and water transport capacity covary with each other in a coordinated manner; this allowed species occupying similar hydrologic environment to have different trait combinations. First, only 13 out of 36 pair-wise correlations between traits were statistically significant (Fig. 2a). Second, three upland species had different trait combinations, as shown in the biplot of the trait PCA (Fig. 2b). Finally, while the water table depth at which the species had peak natural abundance, water table depth optima predicted by the best aster model and the first principal component of the trait PCA were phylogenetically conserved, individual traits were not (Fig. 3).

One pattern that emerged from the trait analyses was that while wetland species had similar combinations of trait values, upland species did not. This pattern may reflect differences in drought tolerance strategies among upland species. Traits examined in this study could be separated into two groups based on their correlations, so that traits within groups were more correlated with each

other than with traits in the other group (Fig. 2a, b). One group of traits included turgor loss point, stomatal pore index, root elongation rate, wood density, and lenticel density on main stems; the other group consisted largely of traits related to gas exchange and hydraulic conductance. One upland species, *S. humilis*, had similar values of the second group of traits as wetland species, but more extreme values in the first group of traits; in contrast, *S. interior* had similar values of the first group of traits as wetland species, but more extreme values in the second group of traits (Fig. 3a). High gas exchange rates and hydraulic conductance allow plants to respond quickly to rainfall events, taking advantage of temporarily available water supply (Maherali and DeLucia 2000, Maherali et al. 2004). Compared to *S. humilis*, *S. interior* may have a drought-avoidance rather than a drought-resistance strategy.

There may be a continuum in the degree of integration among functionally associated traits (Schlichting 1989, Pigliucci 2003). At one end of the continuum, traits may be highly correlated with each other and covary in a coordinated manner, such that these traits can be treated as a single trait syndrome (e.g., Reich 2014). At the other end of the continuum, traits may be uncoupled from each other, which allows species with different multi-trait phenotypes to show the same performance response to environmental factors. Where a given set of traits falls along this continuum may vary among species (e.g., Armbruster et al. 1999). Among the Salicaceae species examined in this study, wetland species showed similar traits combinations while upland species did not.

The result that none of the individual traits were phylogenetically conserved suggested that phylogenetic conservatism in species hydrologic niches was unlikely to be caused by genetic or structural constraints in any of the individual traits. Otherwise, we would observe a significant phylogenetic signal in at least some of the traits. Instead, the phylogenetic signal in hydrologic niches was likely caused by conservatism in the integrated water stress tolerance of species determined by their trait combinations, as indicated by the significant phylogenetic signal found in the first principal component of the trait PCA (Fig. 3b). One caveat is that we only sampled a small subset of species in the Salicaceae family and may lack power to detect phylogenetic signal in traits. A more extensive sampling of species is required to confirm whether or not these results represent the evolution pattern of the traits underlying hydrologic niche and water stress tolerance in the Salicaceae family.

If traits associated with tolerance to the same stress factor do not covary with each other in a coordinated manner, there could be multiple traits combinations that confer the same level of stress tolerance, which can present several challenges for the use of trait-based or phylogenetic approaches to test community assembly processes. One challenge is that it requires the inclusion of multiple traits to predict individual performance (Marks and Lechowicz 2006, Sterck et al. 2011, 2014,

Kraft et al. 2015b, Laughlin and Messier 2015, Schroeder-Georgi et al. 2016). In this study, although seven out of nine traits showed a significant correlation with species natural distributions along the hydrologic gradient (Appendix S1: Table S2), these traits all explained less variation in the water table depth at which species had peak natural abundance than the first principal component of trait PCA and the optimal water table depth. Additionally, many-to-one relationships between trait combinations and stress tolerance could complicate the interpretation of community phylogenetic patterns (Cavender-Bares et al. 2009, Gerhold et al. 2015). For instance, when closely related species have evolved different strategies to solve the same ecological problems (e.g., Schwilk and Ackerly 2001, Agrawal and Fishbein 2006), they may diverge in individual traits while maintaining similar ecological niches, which would lead to phylogenetic clustering without phenotypic clustering in individual traits. Among the Salicaceae species, the degree of integration among traits associated with drought and flooding tolerance varied among species: only upland species had different combinations of traits while wetland species did not. Furthermore, wetland species were more phylogenetically clustered than upland species (Fig. 3a). Consequently, the Salicaceae communities at the wetter end of the hydrologic gradient were more functionally similar and phylogenetically clustered than those in uplands (Savage and Cavender-Bares 2012).

CONCLUSIONS

Our experimental test of fitness differences among willow and poplar species across a hydrologic gradient highlights the importance of environmental filtering in community assembly. The controlled field experimental approach allowed us to detect fitness variation among species in response to the hydrologic environment in the absence of possible confounding effects of dispersal and biotic interactions. This study also reveals that some of the species with contrasting trait combinations had similar hydrologic niches and similar integrated water stress tolerance. While species' natural distributions across the hydrologic gradient were phylogenetically conserved, individual traits associated with water transport capacity or water stress tolerance were not. Phylogenetic conservatism in species niches, in contrast to the lability we found in individual functional traits, suggests that the phylogenetic signal in hydrologic niches is not a consequence of conservatism in individual traits, but rather, conservatism in the integrated stress tolerance of species determined by the combination of multiple traits. By merging a field experiment with functional traits and species distribution data, our study provides an example of integrating multiple approaches in the investigation of environmental filtering.

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Supporting Information

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