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# Habitat specialization and the role of trait lability in structuring diverse willow (genus *Salix*) communities

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*Abstract*. The co-occurrence of closely related species is challenging to explain because biotic filters are expected to limit the ecological similarity of species within communities. To investigate the mechanisms important in facilitating species' co-occurrence in diverse willow and poplar communities, we examined functional diversity and community phylogenetic structure along a hydrologic gradient. We focused on traits related to drought tolerance, leaf hydraulics, and recruitment, and examined species' phylogenetic relatedness and trait lability using a molecular phylogeny. Within habitats, species exhibited phenotypic clustering, and across the landscape, species distributions were correlated with their functional traits in a manner consistent with environmental filtering. With increasing water availability, communities changed from being phylogenetically even to being phylogenetically clustered. We suggest that this shift results from environmental filtering acting on conserved traits in wet habitats and labile traits in dry habitats. Taken together, these results suggest that environmental filtering is important to community assembly along the entire hydrologic gradient within this system. Although many of the traits important to habitat specialization in upland habitats are phylogenetically labile, species' habitat affinity is phylogenetically conserved overall, indicating that niche conservatism can occur as an emergent property despite trait lability. This study demonstrates the complementary nature of trait and community phylogenetic analyses and how these methods can be used to better understand the processes involved in community assembly along environmental gradients.

*Key words: community phylogenetics; drought; functional diversity; phylogenetic signal;* Populus; Salix; *waterlogging.*

## INTRODUCTION

It is frequently hypothesized that closely related species will rarely co-occur because of biotic limitations on ecological similarity within communities. This hypothesis was pioneered by Darwin (1859), who predicted that closely related species should compete heavily for the same resources and exclude one another from communities. While there is evidence that closely related plant species can compete heavily for shared resources (Burns and Strauss 2011, Violle et al. 2011), their co-occurrence may also be limited by a variety of processes including pest–host (Gilbert and Webb 2007) and pollinator–host interactions (Sargent and Ackerly 2008). Abiotic forces also drive plant community structure, as recognized early on by the Danish scientist, Eugenius Warming (1895), who observed that phentopically similar plants are often found in similar environments. More recent work has demonstrated that community assembly can be the result of the combined effects of both biotic (e.g., competition, pest–host interactions, pollinator–host interactions, and mutualism) and abiotic factors (Weiher et al. 1998; reviewed in Cavender-Bares et al. [2009] and Vamosi et al. [2009]). However, the extent that different processes lead to predictable patterns of co-occurrence among closely related species depends on whether shared ancestry is important in determining species' ecological similarity (Prinzing et al. 2001, Losos 2008, Wiens et al. 2010). What remains to be determined is whether the co-occurrence of closely related species is primarily restricted by limiting similarity, or whether their assembly is the result of multiple factors acting on traits that can differ in their phylogenetic lability.

A commonly used method to study community assembly is to compare phylogenetic community structure and patterns of trait phylogenetic signal (Webb et al. 2002, Cavender-Bares et al. 2004*a*, Kraft et al. 2007). This approach is based on the premise that communities are assembled by processes that create predictable patterns of community structure such that closely related species co-occur more (phylogenetic clustering) or less (phylogenetic evenness) often than expected by chance. For example, an environmental condition such as low water availability can act as a filter, favoring the

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co-occurrence of species with similar traits (e.g., high drought tolerance). If closely related species share these traits, then species will be phylogenetically clustered, and if they have divergent traits, then they will be phylogenetically even. For example, Cavender-Bares et al. (2004*a*) found that in Floridian oak communities, traits related to habitat specialization were convergent, likely because they evolved through a process of parallel adaptive radiation. They concluded that environmental filtering on these traits contributed to the significant pattern of phylogenetic evenness in the system.

Both the processes involved in and the traits important to community assembly can be affected by abiotic conditions and can change along environmental gradients. For example, Grime (1977) hypothesized that competition may increase with productivity and decrease with disturbance and environmental stress. This type of change can result in a correlation between phylogenetic community structure and environmental conditions if the traits important to both of these processes have a similar phylogenetic signal. Patterns of phylogenetic community structure can shift across an environmental gradient either due to shifts in dominant assembly processes or to differences in the phylogenetic lability of traits critical to assembly at opposite ends of an environmental gradient. Several studies have examined variation in phylogenetic community structure under different environmental conditions (Verdu and Pausas 2007, Anderson et al. 2011, Kluge and Kessler 2011), including other studies in this issue (Cavender-Bares and Reich 2012, Graham et al. 2012). For example, Graham et al. (2012) investigated hummingbird communities along an elevation gradient and found that hummingbirds are more phylogenetically clustered in harsher, higher elevation habitats. Since ecologically important traits in this clade are phylogenetically conserved, this pattern suggests that environmental filtering increases in importance with elevation. These results are consistent with those found in Serengeti plant communities and fern communities in Costa Rica, where there is greater phylogenetic clustering in stressful habitats (Anderson et al. 2011, Kluge and Kessler 2011). However, environmental gradients do not always lead to changes in phylogenetic community structure (Bryant et al. 2008), especially in cases when community assembly is strongly dominated by one assembly process (Willis et al. 2010).

Multiple studies have found that phylogenetic signal changes with the taxonomic and geographic scale of the analysis (Cavender-Bares et al. 2006, Silvertown et al. 2006, Swenson et al. 2007). In general, processes that limit functional similarity tend to be more influential at smaller spatial scales among more closely related species. This is consistent with the findings of several studies that examined assembly processes in communities dominated by single lineages (Losos et al. 2003, Cavender-Bares et al. 2004*a*, Fine et al. 2005, Slingsby and Verboom 2006).

In central Minnesota, USA, 15 species in the family Salicaceae occur across the landscape, of which 13 are in the genus *Salix* (willows) and two are in the genus *Populus* (poplars). In this study, we investigated the processes that influence community assembly and consider the mechanisms that could facilitate the co-occurrence of willow and poplars species. While this study focuses on a group of closely related species, it differs from other studies because it does not deal with a lineage that has likely undergone in situ adaptive radiation. Salicaceous communities in central Minnesota assembled after the last glaciation (10 000 years ago), and therefore, any patterns observed should reflect recent assembly processes.

Salicaceous species present an interesting system for studying community assembly because they span a hydrologic gradient from sandy prairies to perennially saturated wetlands. This gradient allows us to examine patterns of co-occurrence along an ecologically important gradient. While stress gradients are often considered unidirectional, the hydrologic gradient in this system is more complex because plants experience stress at both ends of the gradient (i.e., waterlogging and drought). Both of these stresses are known to limit the growth and productivity of salicaceous species (Amlin and Rood 2002), and therefore, we hypothesized that environmental filtering will be strong on both ends of the gradient. By extension, we predicted that phenotypic traits will be clustered within communities, and phylogenetic community structure will depend on the lability of traits important to specialization along the gradient.

This study integrates functional trait (Weiher et al. 1998, Cornwell and Ackerly 2009) and community phylogenetic approaches (Webb et al. 2002), and considers the importance of testing trait-based hypotheses. We measured traits associated with drought tolerance, plant hydraulics, and recruitment that we hypothesized are mechanistically linked to assembly processes (Table 1). We also examined two traits related to growth strategies: specific leaf area and relative growth rate, which are ecologically informative in many systems (Reich et al. 2003). The main objectives of our study were to (1) consider the extent that phylogenetic relatedness impacts the assembly of closely related species, (2) test for evidence of environmental filtering and limiting similarity along a hydrologic gradient using functional trait and community phylogenetic approaches, and (3) examine the extent that these approaches can increase our understanding of community assembly in a system with potentially complex species–environment interactions. Since willows are known to hybridize (Brunsfeld et al. 1991, Argus 1997), we also considered how gene flow may have affected patterns of trait lability in the genus.

## METHODS

### *Plant community composition and water availability*

We established 50 $10 \times 30$ m plots in three preserves in eastern Minnesota, USA: Cedar Creek Ecosystem

Table 1. Predicted relationships between water availability and plant functional traits.

| Trait | Wet habitats | Dry habitats | Functional explanation | Source |
|---|---|---|---|---|
| Turgor loss point (TLP) | less negative | more negative | With a more negative TLP, leaves can function at lower water potentials. | Lenz et al. (2006) |
| Wood density (WD) | low | high | High WD helps maintain xylem integrity at low water potentials. | Hacke et al. (2001) |
| Stomatal pore index (SPI)† | high | low | Low SPI can minimize passive water loss through the stomata. | Farquhar and Sharkey (1982) |
| Length of seed viability (LSV) | short | long | Longer viability may facilitate establishment in sites with sporadic water availability. | Vansplunder et al. (1995) |
| Root elongation rate (RER) | slow | fast | Fast root elongation will allow for access to deep water supplies. | Markesteijn and Poorter (2009) |

† Based on the stomatal pore index (Sack et al. 2003).

Science Reserve (part of the Long Term Ecological Research Network, LTER; 45.41° N, −93.19° W), Helen Allison Savannah (45.38° N, −93.18° W), and Boot Lake Scientific and Natural Area (45.34° N, −93.11° W) in the summer of 2007. Plots were randomly selected, with the exception of five plots that were chosen to increase the sampling of rare species. There were 13 *Salix* and 2 *Populus* species in the study area (Table 2). We characterized species' abundance based on total basal area. When plants had fewer than 12 stems, we measured each stem; otherwise, we estimated basal area based on stem count. Total basal area was normalized across all plots for each species to make shrub and tree species more comparable. Water availability was quantified by measuring depth to the water table (WT) monthly in a well in the center of each plot for two growing seasons, May–October (Savage and Cavender-Bares 2011). When there was flooding, we measured the depth of the water above the soil (positive WT). Each plot was considered a plant community in subsequent analyses.

### *Community null models*

We used two null models that randomize species' co-occurrence: one that maintains species richness (Null 1); and one that maintains both species richness and occurrence frequency (Null 2). The first null model is applicable when there is minimal dispersal limitation and all species are equally likely to colonize a community (but see Kembel 2009). Null 2, in contrast, allows species' prevalence to impact their likelihood of colonization. We hypothesized that willow and poplar species are not dispersal limited because their seeds are readily dispersed by wind and water (Gage and Cooper 2005), but we included Null 2 to serve as a more conservative test of community assembly. One drawback of Null 2 is that it is insensitive to situations where rare species (e.g., habitat specialists) co-occur in low species rich communities (Helmus et al. 2007). Unless otherwise mentioned, all analyses were completed using picante version 1.3 (Kembel et al. 2010), vegan version 1.17 (Oksanen et al. 2011), and ape version 2.7 (Paradis et al. 2004) packages in R. In these packages, Null 1 is the "richness" model, and Null 2 is the "independentswap" model. Significance was determined based on 999 randomizations of the community–species matrix.

### *Phylogenetic analysis*

We estimated a phylogeny using the nuclear alcohol dehydrogenase gene (*ADH*) and five chloroplast barcoding genes (*matK*, *rbcLa*, *trnH-psbA*, *atpF-atpH*, and *psbK-psbl*). The *ADH* sequencing was completed in our laboratory using primers obtained from I. Belyaeva et al. (*personal communication*), and the remaining sequences were acquired from the barcode of life data system (Ratnasingham and Hebert 2007). A detailed description of the sample collection, molecular procedures, and phylogenetic analyses are included in Appendix A and Appendix B. We used maximum-likelihood optimality criteria for our analyses and transformed the trees using nonparametric rate smoothing. For the *ADH* analysis, we cloned 7–12 copies of the known polypoid species (Table 2) and examined whether there was evidence of allopolyploid ancestry. We tested the sensitivity of our results to the 16 derivative phylogenies that represented all possible allele combi-

Table 2. Willow (*Salix*) and poplar (*Populus*) species and their distributions across a hydrologic gradient in central Minnesota, USA.

| Species | $WT_{wet}$ | $WT_{dry}$ |
|---|---|---|
| *P. deltoides* Bartram ex Marsh. | −0.77 | −0.86 |
| *P. tremuloides* Michx. | −0.82 | −1.04 |
| *S. amygdaloides* Andersson | −0.19 | −0.40 |
| *S. bebbiana* Sarg. | −0.18 | −0.46 |
| *S. candida* Flueggé ex Willd. | 0.01 | −0.30 |
| *S. discolor* Muhl.† | −0.03 | −0.50 |
| *S. eriocephala* Michx. | −0.18 | −0.62 |
| *S. humilis* Marsh.† | −1.02 | −1.10 |
| *S. interior* Rowlee | −0.83 | −1.17 |
| *S. lucida* Muhl.† | 0.07 | 0.00 |
| *S. nigra* Marsh. | −0.01 | −0.24 |
| *S. pedicellaris* Pursh | 0.04 | −0.14 |
| *S. petiolaris* Sm. | −0.15 | −0.43 |
| *S. pyrifolia* Andersson | 0.01 | −0.04 |
| *S. serissima* (L.H. Bailey) Fernald† | 0.02 | −0.20 |

*Notes:* Species distributions are described in terms of their weighted average depth to the water table (m) in the wettest ($WT_{wet}$) and the driest month ($WT_{dry}$) of the year. Polyploid species are marked with a dagger (†).

nations of the *ADH* gene and a phylogeny estimated based on all six genes.

### *Trait–environment correlations*

We tested for trait–environment relationships based on a priori hypotheses (Table 1) at the species and community level to examine evidence for environmental filtering. Traits were measured in the field and a greenhouse common garden, and methods are described in Appendix C. First, we compared species' mean trait values with their habitat affinities, which were characterized by mean depth to the water table in August, the driest month of the year ($\mathrm{WT}_{\mathrm{dry}}$), weighted by species' normalized basal area within communities. Weighted $\mathrm{WT}_{\mathrm{dry}}$ describes the environmental conditions where each species grows the most vigorously (Cavender-Bares et al. 2004*b*). Second, we examined whether traits are associated with habitat affinity across the phylogeny using phylogenetically independent contrasts (Felsenstein 1985). Third, we tested whether there was a relationship between mean trait values and $\mathrm{WT}_{\mathrm{dry}}$ within communities. We weighted mean trait values by species abundance to account for differences in species' prevalence within and frequency across communities. We analyzed all three of these relationships using least-squares regression.

### *Phenotypic clustering and functional diversity*

On a univariate level, we examined phenotypic clustering within communities by testing for a correlation between species' pairwise co-occurrence (Schoener 1970) and their trait differences using least-squares regression and compared our results with the two null models. A negative correlation indicates phenotypic clustering and a positive correlation indicates phenotypic evenness. On a multivariate level, we quantified functional diversity based on three metrics (Fig. 1): functional richness ($F_{\text{ric}}$; Cornwell et al. 2006), functional evenness ($F_{\text{eve}}$), and functional divergence ($F_{\text{div}}$; Villeger et al. 2008). We selected these metrics because they are independent and have sufficient power to distinguish both limiting similarity and filtering from neutral processes (Mouchet et al. 2010). To avoid using the same traits twice in this analysis, we only used field measurements (with the exception of traits that were only measured in the greenhouse). We calculated functional diversity using the FD package in R (Laliberte and Legendre 2010). We considered metrics to be significant when they were in the extreme 2.5% of the null distributions (Null 1 and 2). The expectation is that limiting similarity should lead to high functional diversity (high $F_{\text{eve}}$, $F_{\text{ric}}$, $F_{\text{div}}$) and filtering should lead to low functional diversity.

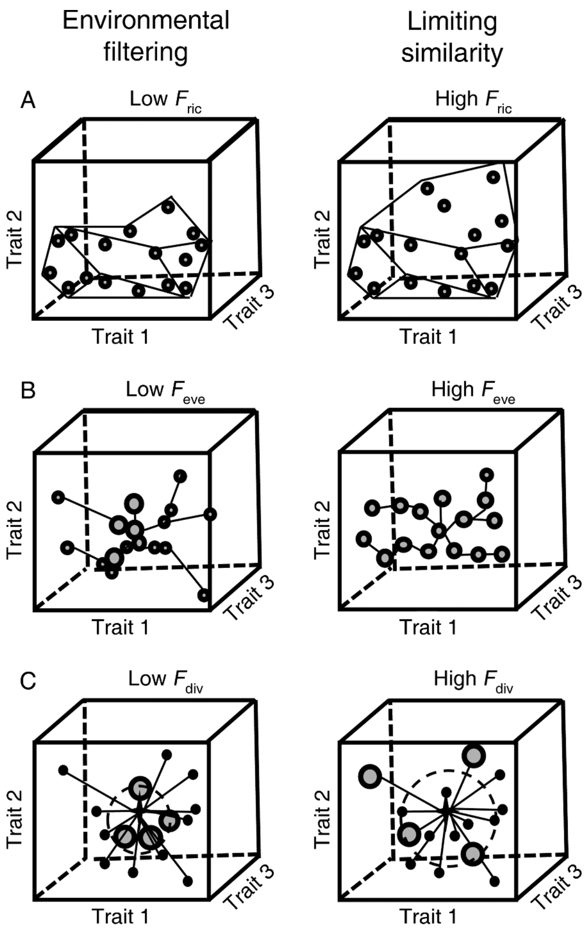

FIG. 1. Three multivariate indices for measuring functional diversity within communities. (A) Functional richness ($F_{\text{ric}}$) is a measure of the volume of functional area occupied by species in *n*-dimensional trait space. $F_{\text{ric}}$ is higher when the volume of the convex hull is larger (Cornwell et al. 2006). (B) Functional evenness ($F_{\text{eve}}$) is a measure of the regularity of species' distribution and abundance in trait space. This is determined based on the minimum spanning tree (MST) of the species. $F_{\text{eve}}$ is greater when traits are evenly distributed along the MST and species have similar abundances. (C) Functional divergence ($F_{\text{div}}$) is a measure of how species' abundance is distributed in trait space. $F_{\text{div}}$ is greater when the most abundant species occur far away from the center of gravity (Villeger et al. 2008). The mean distance from the center of gravity is indicated by a circle. The prediction is that environmental filtering will cause low $F_{\text{ric}}$, $F_{\text{eve}}$, and $F_{\text{div}}$, and limiting similarity will cause high $F_{\text{ric}}$, $F_{\text{eve}}$, and $F_{\text{div}}$. The figure is modified from Villeger et al. (2008).

### *Changes in phenotypic clustering along a gradient*

Using both univariate and multivariate approaches, we examined whether trait similarity within communities changed along a water availability gradient. On a univariate level, we examined whether mean weighted deviation for each trait varied with water availability. This metric was calculated as:

$$\frac{2}{\pi}\arctan\left(5\left[\sum_{i=1}^{N} w_i(\ln x_i - \ln x)^2\right]\right)$$

where $x_i$ is the trait average for the species $i$, $\ln x$ is the weighted logarithmic mean of the trait, and $w_i$ is the relative abundance of the species $i$ in the community (Mason et al. 2003). A low deviation indicates phenotypic clustering and a high deviation indicates phenotypic evenness. On a multivariate level, we examined the relationship between functional diversity ($F_{\text{eve}}$, $F_{\text{ric}}$, and $F_{\text{div}}$) and $\text{WT}_{\text{dry}}$. We examined both univariate and multivariate data using quantile regression and determined its significance using the rank test statistic ($\alpha = 0.05$) for quantiles 0.1, 0.25, 0.50, 0.75, and 0.90. Analysis was completed using quantreg version 4.71 in R (Koenker 2011).

### *Trait phylogenetic signal*

We examined the phylogenetic signal of species' traits using the *K* statistic (Blomberg et al. 2003), a metric that determines when trait evolution deviates significantly from a Brownian motion model of evolution. We only used trait data measured in the field because the *K* statistic has low power when there are fewer than 15 species ($N \approx 9$ in the greenhouse). We described species' habitat affinity based on weighted $\text{WT}_{\text{dry}}$ and weighted $\text{WT}_{\text{wet}}$ (depth to water table in wettest month, May) to examine whether habitat affinity exhibited phylogenetic signal. We compared the observed *K* statistics to a null model based on 999 randomizations of the species across the tips of the phylogeny ($K_{\text{null1}}$). We also used a more conservative null model ($K_{\text{null2}}$) that evolved each trait under Brownian motion 999 times using the rTraitCont function in ape with a rate of evolution calculated from the data (using the fitContinuous function in Geiger; Harmon et al. 2009). We compared our results to the top and bottom 2.5% of the two null distributions.

### *Community phylogenetic structure*

The first metric we used was NRI (net relatedness index), which is the standardized size effect of mean pairwise phylogenetic distance between species (Webb 2000). A negative NRI indicates phylogenetic evenness and a positive NRI indicates phylogenetic clustering. We examined whether there was phylogenetic signal in species' abundances because it can impact the power of NRI to detect limiting similarity (Kembel 2009). The second metric we used was PSV (phylogenetic species variability; Helmus et al. 2007), which tests the extent that phylogenetic relatedness within a community decreases the variance of a hypothetical neutral trait evolving under a Brownian motion model. It ranges from 0 to 1, with 1 indicating a community with a star phylogeny and high phylogenetic variability, and 0 representing a community with low variability and a high phylogenetic clustering. This metric is beneficial because it does not linearly correlate with species richness and does not require standardization. We assessed the significance of NRI and PSV using both null models and directly examined the relationship between phylogenetic community structure and water availability by comparing these metrics to $\text{WT}_{\text{dry}}$ using quantile regression. We assessed the significance of the quantile regression using the rank test statistic. We also divided communities into two groups (those that seasonally experienced waterlogging [$\text{WT}_{\text{wet}} \geq 0$ m] and those that did not) and compared the phylogenetic signal of these groups to null expectations.

## RESULTS

### *Trait-based evidence for environmental filtering and limiting similarity*

Most traits varied across a hydrologic gradient in a manner consistent with our a priori hypotheses (Table 1) when analyzed directly (Fig. 2A), and considering phylogenetically independent contrasts (Appendix D: Table D1). These trends were also present when traits were analyzed at the community level (Fig. 2B). Overall, species from drier habitats had lower turgor loss points (TLP; in the greenhouse, $F = 32.4$, $P < 0.001$, and in the field, $F = 13.0$, $P < 0.01$), and higher wood density (WD; in the field, $F = 5.2$, $P < 0.05$), which is consistent with a higher drought tolerance. Traits related to recruitment also varied as predicted, with the length of seed viability (LSV) being negatively correlated with ($F = 7.6$, $P < 0.05$) and root elongation rate (RER) being positively correlated with ($F = 20.1$, $P < 0.001$) water availability. The remaining traits either demonstrated no relationship with water availability (specific leaf area, SLA, and relative growth rate, RGR) or exhibited a pattern contrary to our expectations (stomatal pore index, SPI). For example, SPI was negatively correlated with water availability when measured in the greenhouse ($F = 17.2$, $P = 0.004$) and the field ($F = 9.1$, $P = 0.01$; Fig. 2A). Traits also varied in their constraint along a hydrologic gradient. Several traits associated with specialization in dry habitats (TLP, WD, and RER) demonstrated lower mean deviation within communities in these habitats, while SPI and LSV (only when $\tau = 0.90$) had lower variability in wet habitats (Fig. 2C).

Overall, communities had a lower functional richness ($F_{\text{ric}}$) then expected by chance (based on Null 1 but not Null 2; Table 3). This is consistent with the phenotypic clustering observed for TLP, WD, SPI, and RER within communities (Fig. 3, Table 4). Across plots, there was also a significant negative correlation between $F_{\text{ric}}$ and water availability, indicating that communities in wetter habitats tended to be more phenotypically clustered (Fig. 5A). Meanwhile, functional divergence ($F_{\text{div}}$) did not deviate from null expectations, and functional evenness ($F_{\text{eve}}$) was greater than expected by Null 2 (Table 4).

### *Phylogenetic analyses*

According to the *ADH* phylogeny and the six-gene phylogeny, willows and poplars break into two monophyletic groups (Fig. 4). *Salix nigra*, *S. amygdaloides*, *S. serissima*, and *S. lucida* form well-supported clades (clades c and e) in both phylogenies, consistent with the subgenus *Salix* (Argus 1997). Two of the polyploid species, *S. lucida* and *S. serissima*, appear to be allopolyploids and contain alleles from different clades (clades a and c; Fig. 4A). The ancestry of the remaining

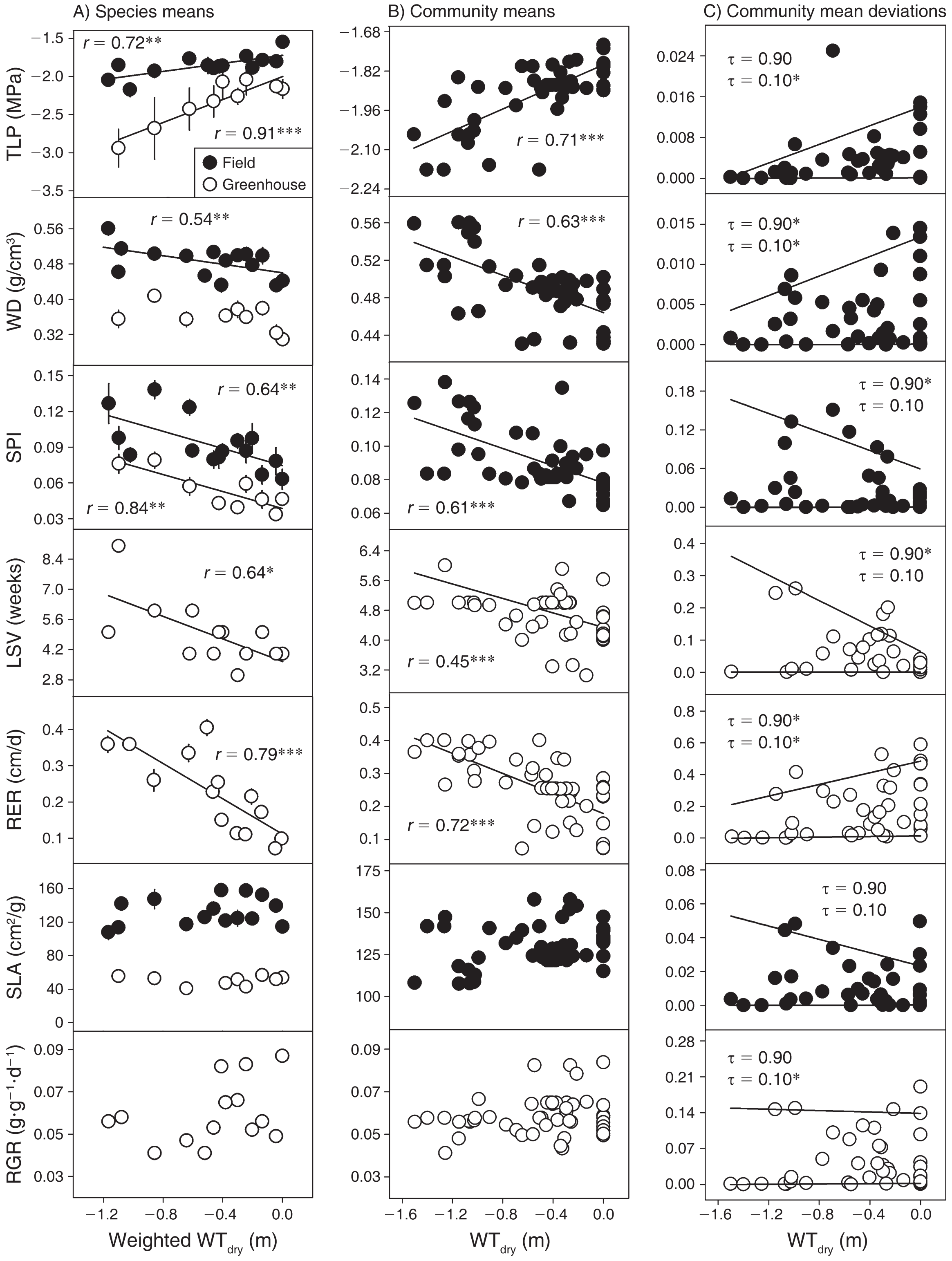

FIG. 2. (A) Correlations between functional traits and species' habitat affinities across a hydrologic gradient, and (B) between weighted community means and water availability at each site. Water availability is described based on the depth to the water table in the driest month ($WT_{dry}$) and is weighted by species' abundance. Traits are as follows: TLP, turgor loss point; WD, wood density; SPI, stomatal pore index; LSV, length of seed viability; RER, root elongation rate; SLA, specific leaf area; and RGR, relative growth rate. Error bars show ±SE. (C) Variation in trait deviation within communities along a hydrologic gradient. Traits measured in the field are marked with closed circles, and traits measured in a greenhouse are marked with open circles. Significant quantile regressions are indicated with asterisks.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 3. Mean functional richness ($F_{\mathrm{ric}}$), evenness ($F_{\mathrm{eve}}$), and divergence ($F_{\mathrm{div}}$) within communities.

| Metric | Mean | CI (Null 1) | CI (Null 2) |
|---|---|---|---|
| $F_{\mathrm{ric}}$ | 0.16 | 0.17–0.21* | 0.14–0.18 |
| $F_{\mathrm{eve}}$ | 0.60 | 0.48–0.62 | 0.38–0.54* |
| $F_{\mathrm{div}}$ | 0.83 | 0.70–0.83 | 0.78–0.87 |

*Notes:* The 90% confidence interval (CI) is reported for two null models (described in *Methods*). Asterisks note significant values ($\alpha = 0.05$).

polyploids (*S. discolor* and *S. humilis*) is less clear. These species were paraphyletic in clades that were both poorly supported (clades a and b). Taken together, these data demonstrate that there is evidence of reticulate evolution in the genus *Salix*.

### *Trait lability*

Only two of the measured functional traits (SLA and RGR) exhibited a greater phylogenetic signal than expected based on $K_{\mathrm{null1}}$ (Table 5). This signal was only significant considering some of the *ADH* phylogenies and not the six-gene phylogeny. However, species' habitat affinity ($\mathrm{WT}_{\mathrm{dry}}$) demonstrated a high phylogenetic signal considering $K_{\mathrm{null1}}$ and multiple phylogenetic hypotheses. Considering $K_{\mathrm{null2}}$, none of the traits or habitat preferences significantly exceeded the values evolved under Brownian motion, but two of the traits (WD and SPI) were significantly more labile than expected based on this null model.

### *A shift in phylogenetic community structure*

Communities in wetter habitats exhibited a higher net relatedness index (NRI) and a lower phylogenetic species variability (PSV), indicating greater phylogenetically clustering in these habitats (Fig. 5B, C). Additionally, dry communities demonstrated more variability in their phylogenetic community structure. This was apparent from the increase in slope in the higher quantiles of the regression analysis involving NRI and water availability (Appendix D: Table D2). For PSV, the slope of the $\tau = 0.75$ was significantly greater than $\tau = 0.25$ when considering the six-gene phylogeny (Fig. 5B), but not all of the *ADH* phylogenies. Twenty-six communities demonstrated significant phylogenetic clustering, and between 11 and 13 demonstrated significant phylogenetic evenness based on PSV and considering the six-gene phylogeny. The majority of clustered communities were either perennially saturated or had a $\mathrm{WT}_{\mathrm{dry}}$ >–30 cm. However, NRI yielded less significant results than PSV, with only one community demonstrating a higher NRI (Null 1 and 2), and one demonstrating a lower NRI (Null 2) than expected by chance. More of

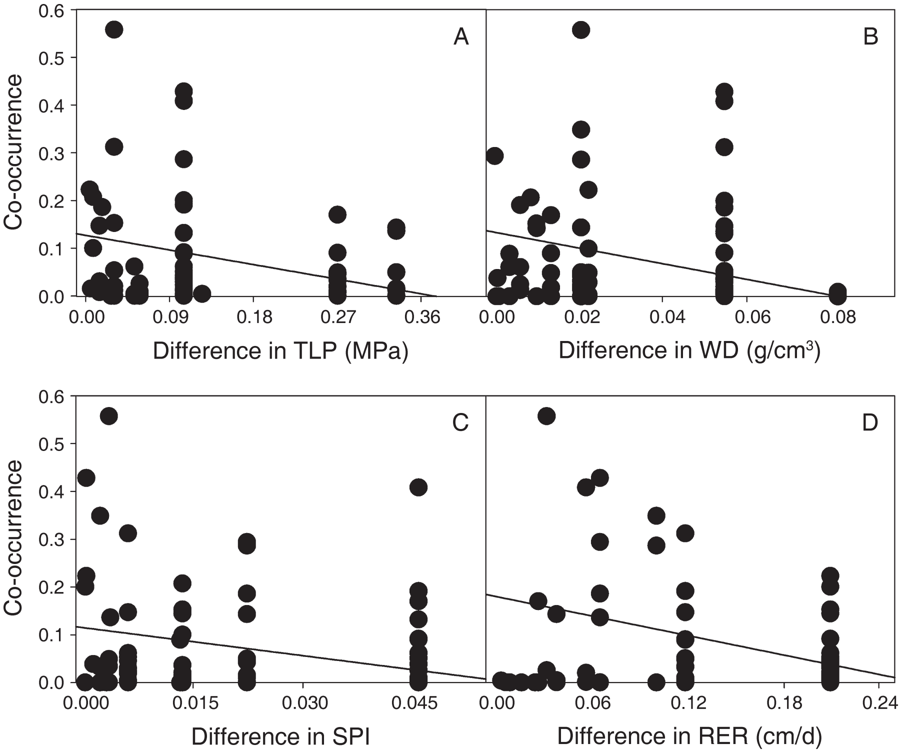

FIG. 3. Correlations between species' pairwise co-occurrence and (A) leaf turgor loss point (TLP), (B) wood density (WD), (C) stomatal pore index (SPI), and (D) root elongation rate (RER). Field measurements are reported for TLP, WD, and SPI. Species' co-occurrence was determined based on Schoener's (1970) co-occurrence index. All correlations were significant based on a 0.75 quantile regression analysis ($\alpha = 0.05$).

TABLE 4. Slopes and *P* values for quantile regression analyses ($\tau = 0.75$) of species' pairwise trait difference on their level of co-occurrence (see Fig. 3) using two different null models (described in *Methods*).

| | Field | | | Greenhouse | | |
|---|---|---|---|---|---|---|
| Trait | Slope | Null 1 | Null 2 | Slope | Null 1 | Null 2 |
| Turgor loss point | −0.34 | 0.02* | 0.06 | −0.06 | 0.27 | 0.47 |
| Wood density | −1.65 | 0.01* | 0.02* | −2.20 | 0.05* | 0.04* |
| Stomatal pore index | −1.95 | 0.04* | 0.15 | −4.24 | 0.03* | 0.16 |
| Length of seed viability | NA | NA | NA | 0.01 | 0.72 | 0.83 |
| Root elongation rate | NA | NA | NA | −0.66 | 0.01* | 0.02* |
| Specific leaf area | 0.00 | 0.31 | 0.29 | 0.00 | 0.31 | 0.38 |
| Relative growth rate | NA | NA | NA | −1.87 | 0.14 | 0.42 |

*Notes:* Traits were measured in the field and the greenhouse. Significant *P* values are marked with an asterisk ($\alpha = 0.05$).

the communities exhibited phylogenetic structure considering the *ADH* phylogenies (Appendix D: Table D3).

Communities that experienced waterlogging tended to have a lower mean phylogenetic distance (MPD = 221.3) than those that did not (MPD = 306.7), but this pattern was only significant considering NRI and Null 1. Species' abundances did not demonstrate a significant phylogenetic signal, and there was no relationship between spatial distance between plots and species composition based on a Mantel test (data not shown). We also tested the effect of removing the two poplar species from the study to examine whether they were biasing the results. With these species excluded, there was still a relationship between water availability and phylogenetic structure considering NRI but not PSV (Appendix D: Table D4).

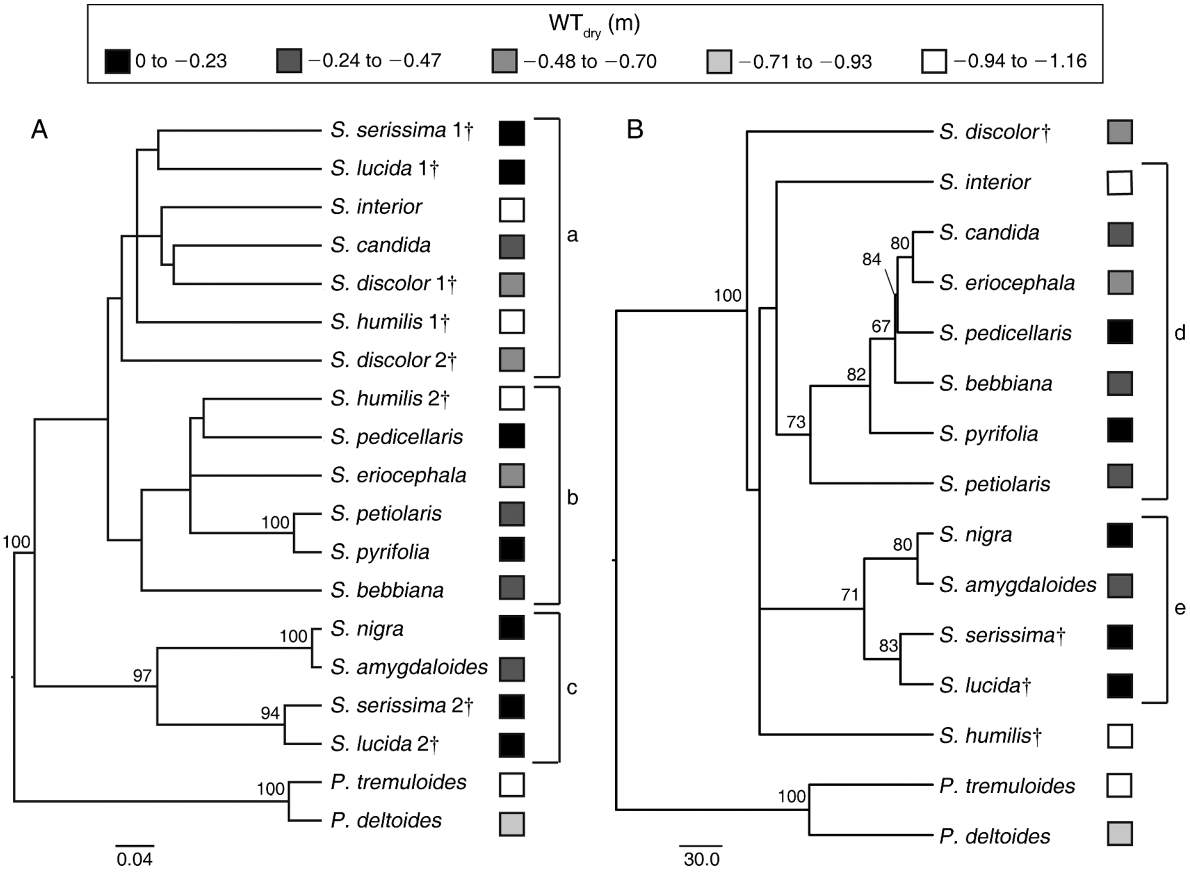

FIG. 4. Habitat affinity mapped onto (A) the *ADH* phylogeny and (B) the six-gene phylogeny (*ADH*, *matK*, *rbcLa*, *trnH-psbA*, *atpF-atpH*, and *psbK-psbI*) of 15 willow (*Salix*) and poplar (*Populus*) species. Species' habitat affinities are described by mean weighted depth to the water table in the driest month (WT$_{dry}$). Phylogenies were estimated using maximum-likelihood optimality criterion and nonparametric rate smoothing. The lowercase letters indicate the major clades that are apparent based on the phylogenetic analyses. Bootstrap values are shown for all nodes that have over 50% support. Polyploid species are marked with a dagger (†). Further details on phylogenetic analyses are in Appendices A and B.

TABLE 5. Phylogenetic signal of phenotypic traits and habitat affinities.

| | Six-gene phylogeny | | | *ADH* phylogenies | |
|---|---|---|---|---|---|
| Traits | $K_{obs}$ | $\bar{K}_{null1}$ | $\bar{K}_{null2}$ | $\bar{K}_{obs}$ | $P$ |
| $WT_{dry}$ | 0.88* | 0.29* | 1.00 | 0.60* | 0.02–0.16* |
| $WT_{wet}$ | 1.45* | 0.30* | 0.99 | 0.73* | 0.02–0.15* |
| Turgor loss point | 0.47 | 0.35 | 1.02 | 0.37 | 0.09–0.50 |
| Wood density | _0.29*_ | _0.29_ | _1.02*_ | 0.18 | 0.77–0.84 |
| Stomatal pore index | _0.34*_ | _0.30_ | _0.98*_ | 0.35 | 0.13–0.45 |
| Length of seed viability | 0.73 | 0.39 | 1.02 | 0.50 | 0.33–0.43 |
| Root elongation rate | 0.50 | 0.29 | 1.00 | 0.47 | 0.09–0.35 |
| Specific leaf area | 0.43 | 0.29 | 0.99 | 0.74* | 0.01–0.08* |
| Relative growth rate | 0.41 | 0.29 | 1.03 | 0.60* | 0.02–0.22* |

*Notes:* Observed $K$ statistics ($K_{obs}$) are reported for the six-gene phylogeny (Fig. 4B), followed by the means of both null models ($\bar{K}_{null1}$ and $\bar{K}_{null2}$; described in *Methods*). The mean observed $K$ values ($\bar{K}_{obs}$) are reported for the 16 *ADH* phylogenies along with the range of $P$ values under $K_{null1}$. All traits were measured in the field except LSV and RER. Observed $K$ values significantly greater than null expectations are marked with an asterisk ($\alpha = 0.05$). Observed $K$ values significantly less than $K_{null2}$ are shown in italics.

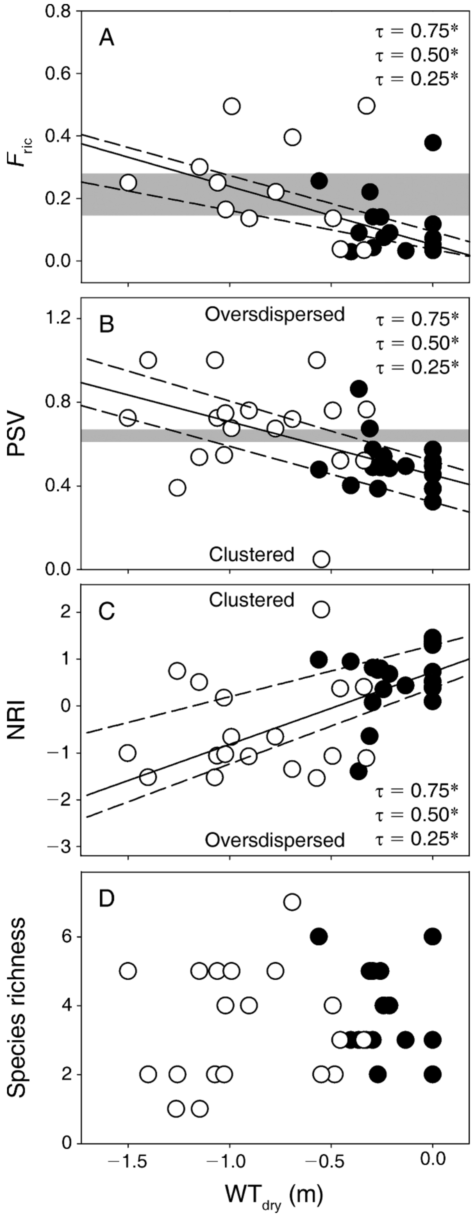

FIG. 5. (A) Correlations between functional richness ($F_{ric}$), (B) phylogenetic species variability (PSV), and (C) net relatedness index (NRI) and water availability ($WT_{dry}$). Quantile regressions are graphed for $\tau = 0.25$ (lower dashed line), 0.50 (solid line), and 0.75 (upper dashed line). Significant slopes ($\alpha = 0.05$) are marked with an asterisk. In panels (A) and (B), the range of values yielded from Null 1 (95% CI) are marked with gray bars. Communities above and below these bars differ significantly from the null. These results are based on the six-gene phylogeny, and correlations based on other phylogenies are described in Appendix D: Table D2. (D) Species richness across the water availability gradient. In all graphs, communities that experience waterlogging in the spring are marked with solid circles, and the remaining communities are marked with open circles.

## DISCUSSION

Despite the prediction that closely related species should demonstrate limited co-occurrence because of their shared ancestry (Darwin 1859, MacArthur and Levins 1967, Gilbert and Webb 2007), many species in the family Salicaceae co-occur within communities in central Minnesota. Based on both trait and community phylogenetic data, we infer that community assembly is a result of multiple assembly processes that act on traits that differ in their phylogenetic lability. We suggest that environmental filtering acts on phylogenetically labile traits in dry habitats and on conserved traits in wet habitats, causing a change in phylogenetic community structure along this gradient. This pattern contrasts that found in Floridian oak communities, where traits critical for habitat specialization tend to be convergent regardless of habitat (Cavender-Bares et al. 2004*a*), likely due to parallel adaptive radiation.

In this study, there is evidence that environmental filtering plays a dominant role in community assembly based on the strength of trait–environment relationships both at the species and community level (Fig. 2). These trends are further supported by phenotypic clustering (Table 4, Fig. 3) and low functional richness within communities (Table 3). In general, species from drier habitats had traits associated with higher drought tolerance and traits important to establishing in areas with limited water availability (Fig. 2). There were two traits that deviated from our expectations. First, stomatal pore index decreased instead of increasing with water availability. This pattern may indicate that low stomatal area is beneficial in wet habitats because it reduces the water stress imposed by waterlogging. Second, length of seed viability was not phenotypically clustered within communities despite its strong association with water availability (Fig. 2A, Table 3). This pattern suggests that, although having long-lived seeds may increase the likelihood of establishment in dry habitats, it may not be the only successful recruitment strategy in this system (e.g., some species may heavily rely on clonal reproduction; Karrenberg et al. 2002).

It is frequently hypothesized that physiological constraints on xylem anatomy create a trade-off between drought tolerance and growth, which determines species distributions along a hydrologic gradient (Pockman and Sperry 2000). While there is strong evidence for the importance of this trade-off in drier habitats (Hacke and Sperry 2001), it has rarely been examined in wetland communities (except see Luo et al. 2008), and we found no evidence for a relationship between relative growth rate and water availability in this study (Fig. 2A, B). However, drought tolerance was more constrained (less variable) in dry habitats, indicating that filtering on drought tolerance traits was stronger in these habitats (Fig. 2C). The opposite pattern was observed for the one trait, stomatal pore index, that was associated with specialization in wet habitats. These results suggest that the traits important for environmental filtering shift along this hydrologic gradient, and that phenotypic constraint within communities is dependent on the extent that traits are required for specialization within habitats. These results are also consistent with research in wet meadows that suggests there is a trade-off between drought and waterlogging tolerance (Araya et al. 2011).

While we did not measure species' waterlogging tolerance, there is evidence that this tolerance is phylogenetically conserved in some lineages (Waterway et al. 2009). If this is true in the family Salicaceae, filtering on waterlogging tolerance could explain the phylogenetic clustering observed in wet habitats. Meanwhile, the phylogenetic evenness observed in dry habitats could be caused by environmental filtering acting on labile traits (Fig. 5, Table 5). Therefore, changes in the traits involved in habitat specialization could explain the shift in phylogenetic community structure along a hydrologic gradient. One potential complication with this interpretation is the strong phylogenetic signal observed in species' habitat affinities (Table 5). This signal suggests that closely related species are ecologically similar despite the phylogenetic lability observed in many of their functional traits. As a result, it is important to consider alternative explanations for the pattern and examine why habitat affinity might be conserved when traits are predominantly labile. One possibility is that patterns of trait evolution affect the power of our methods to detect phylogenetic clustering within communities. Kraft et al. (2007) demonstrated that environmental filtering on a conserved trait is more likely to create a pattern of phylogenetic clustering when traits are derived instead of ancestral. If affinity toward drier habitats is ancestral in Salicaceae, as suggested by the limited subset of species in this study, then there may be less power to detect phylogenetic clustering in dry habitats. Another option is that the continuous nature of the environmental gradient makes it difficult to separate the effect of species' affinity toward dry vs. wet habitats, and as a result, habitat affinity demonstrates phylogenetic signal when only affinity toward wet habitats is conserved. Lastly, depth to the water table may not capture all the complexities of the plant–environment relationships that the trait data captures because it simplifies species' habitat affinities to one environmental axis (weighted $\mathrm{WT}_{\mathrm{dry}}$). There are several species with similar weighted $\mathrm{WT}_{\mathrm{dry}}$ that rarely co-occur in the field (e.g., *S. interior* and *S. humilis*). This is either an indication of limiting similarity or a result of species specializing along additional unmeasured environmental gradients.

Evidence was mixed regarding the extent to which willow and poplar communities are shaped by limiting similarity. The general expectation is that these processes lead to higher levels of functional diversity than observed in this study (Table 3). However, functional evenness ($F_{\mathrm{eve}}$) was significantly greater than expected based on one null model, suggesting limiting similarity could be important within this system. Additionally, two traits related to species' growth strategies (specific leaf area and relative growth rate) demonstrated phylogenetic signal based on the *ADH* phylogenies (but not the six-gene phylogeny; Table 5). If these traits influence species' competitive abilities, then competition could be important in maintaining phylogenetic evenness in dry habitats. Furthermore, the higher functional richness observed in these habitats could be indicative of niche differentiation (Fig. 5A). Previous research on a subset of willow species suggests that co-occurring species often demonstrate different drought survival strategies, which could lead to differential water use when water is limited (Savage and Cavender-Bares 2011). If this type of niche differentiation is widespread, it could facilitate species' co-occurrence while maintaining strong trait–environment relationships. Therefore, the observed patterns of community phylogenetic structure could result from the combined effects of multiple processes. If waterlogged habitats are more stressful and less productive, a shift in the relative importance of competition along this gradient could be consistent with Grime's (1977) hypothesis. It is also possible that seasonal disturbance from flooding decreases the importance of competition in wet habitats (Connell 1978, Huston 1979).

In interpreting phylogenetic patterns, it is important to realize that lineage sorting and segregation during sexual reproduction can lead to discrepancies between gene trees and the true species' phylogeny (Pamilo and Nei 1988, Doyle 1992). Since there have been relatively few phylogenetic studies of Salicaceae (Leskinen and Alstrom-Rapaport 1999, Azuma et al. 2000), additional research is needed to better understand species' phylogenetic relatedness in this family. It is also evident that willow and poplar diversity is, in part, a product of hybridization and reticulate evolution (Fig. 4). While reticulate evolution has occurred widely across plant lineages, this topic is rarely addressed in studies of this nature. We dealt with the issue of allopolyploidy by testing our results against multiple phylogenetic hypotheses. This simplification was necessary to use current methods, but it is not an accurate representation of allopolyploid genetic history. Therefore, we stress the need for further research on the impact of hybridization on trait evolution and measures of phylogenetic community structure. While reticulate evolution is often viewed as a complicating factor in phylogenetic analyses, it is widespread in the angiosperm phylogeny (Arnold 1997) and may play a critical role in trait evolution. A low level of gene flow could allow for the spread of adaptive alleles between species, facilitate species' rapid diversification (Anderson 1949, Arnold 2004) and potentially account for the high trait lability observed in this study.

Our study demonstrates the insights that can be gained by the combined use of community phylogenetic and functional trait analyses in studies of community assembly. While trait-based analyses reveal information about specific mechanisms, phylogenetic information can provide a broader context for understanding species similarity and help elucidate the relative importance of different processes and functional traits. In this study, the trait data provide strong evidence for environmental filtering, but due to the lack of information on species' waterlogging tolerance, information on community phylogenetic structure was critical for drawing conclusions about filtering in wet habitats. The phylogenetic analyses also reveal that there are complexities in trait evolution and suggest an important role for interspecific gene flow in trait lability. Moreover, habitat affinities are conserved, demonstrating niche conservatism even within this narrow phylogenetic scale and despite lability in particular traits. This outcome reveals that niche conservatism can result as an emergent property despite labile combinations of phenotypic attributes and stress tolerance strategies. These contrasting strategies may promote complementarity and coexistence within upland communities. Taken together, the functional trait and community phylogenetic analyses provide a potential explanation for how different assembly processes can shape communities along a complex environmental gradient.

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## SUPPLEMENTAL MATERIAL

### Appendix A

Phylogenetic and molecular analysis of the *ADH* gene for 15 species in the family Salicaceae (*Ecological Archives* E093-182-A1).

### Appendix B

Phylogenetic and molecular analysis of the five barcoding genes (*matK*, *rbcLa*, *trnH-psbA*, *atpF-atpH*, and *psbK-psbl*) for 15 species in the family Salicaceae (*Ecological Archives* E093-182-A2).

### Appendix C

Functional trait methods and data from the field and greenhouse (*Ecological Archives* E093-182-A3).

### Appendix D

Phylogenetically independent contrasts (PIC), net relatedness index (NRI), and phylogenetic species variability (PSV) results considering different phylogenetic hypotheses (*Ecological Archives* E093-182-A4).