

Contrasting effects of plant species traits and moisture on the decomposition of multiple litter fractions

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Abstract Environmental variation in moisture directly influences plant litter decomposition through effects on microbial activity, and indirectly via plant species traits. Whether the effects of moisture and plant species traits are mutually reinforcing or counteracting during decomposition are unknown. To disentangle the effects of moisture from the effects of species traits that vary with moisture, we decomposed leaf litter from 12 plant species in the willow family (Salicaceae) with different native habitat moisture preferences in paired mesic and wetland plots. We fit litter mass loss data to an exponential decomposition model and estimated the decay rate of the rapidly cycling litter fraction and size of the remaining fraction that decays at a rate approaching zero. Litter traits that covaried with moisture in the species' native habitat significantly influenced the decomposition rate of the rapidly cycling litter fraction, but moisture in the decomposition environment did not. In contrast, for the slowly cycling litter fraction, litter traits that did not covary with moisture in the species' native habitat and moisture in the decomposition environment were

significant. Overall, the effects of moisture and plant species traits on litter decomposition were somewhat reinforcing along a hydrologic gradient that spanned mesic upland to wetland (but not permanently surface-saturated) plots. In this system, plant trait and moisture effects may lead to greater in situ decomposition rates of wetland species compared to upland species; however, plant traits that do not covary with moisture will also influence decomposition of the slowest cycling litter fraction.

Keywords Cedar Creek · Decomposition · Hydrologic gradient · Plant traits · Salicaceae

Introduction

Plant species traits vary predictably along environmental gradients due to environmental filters on species presence (Keddy 1992; Cavender-Bares et al. 2004; Cornwell and Ackerly 2009; Weiher et al. 2011), phenotypic responses to the environment (Sultan 2000; Valladares et al. 2007), and strong relatedness among traits (Ackerly and Reich 1999; Reich et al. 1999; Wright et al. 2004). While the effects of plant species traits on ecosystem processes, such as litter decomposition, have been studied extensively (Cornwell et al. 2008), predicting these processes across environmentally heterogeneous habitats, where both plant species composition and the environment change, remains a challenge. Specifically, whether the effects of environmental conditions on plant species composition and associated traits, as well as the direct effects of environmental conditions, are mutually reinforcing or negating during decomposition is unknown. Are the environmentally determined plant species traits that lead to faster decomposition

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coincident with the environmental conditions that also lead to faster decomposition?

To date, empirical studies that disentangle species trait effects from direct environmental effects have yielded inconsistent results. Using site moisture conditions as an example, Schuur (2001) found that species traits and environmental effects were reinforcing across a very wet precipitation gradient from 2200 to 5050 mm year⁻¹: in addition to directly slowing litter decomposition, high levels of precipitation were associated with high litter lignin concentration, which also slowed decomposition. In contrast, along a precipitation gradient that included very dry sites, ranging from 500 to 5500 mm year⁻¹ of precipitation, Austin and Vitousek (2000) did not find precipitation and lignin effects to be reinforcing: decomposition increased with site precipitation and the positive direct effect of precipitation was larger than the negative effect caused by higher litter lignin in more mesic sites. Further research is needed to clarify how plant traits and the environment influence decomposition across relevant environmental gradients.

Furthermore, trait and environmental effects on litter decomposition may differ between litter fractions that decompose at different rates. Litter is a heterogeneous mixture of biomolecules with differing susceptibilities to microbial decay: the quickly cycling litter fraction is comprised of relatively simple substrates that decompose readily, while more complex substrates that are difficult to degrade, such as lignin, dominate the slowly cycling litter fraction (Berg and McClaugherty 2007). Plant traits can influence the composition and relative sizes of quickly versus slowly cycling litter fractions (e.g., Berg and Ekbohm 1991). Furthermore, the environment may affect the decomposition of these fractions differently. For example, in a global decomposition dataset, Adair et al. (2008) found that the decomposition rate of the slowest cycling litter fraction was less in wetland sites relative to all other sites, but that the quickly cycling fraction decomposed faster in wetland sites. Since lignin decomposition is largely aerobic (Thevenot et al. 2010), this result may be due to anaerobic inhibition of lignin decomposition in the slow cycling fraction in wetlands. Although the majority of litter decomposition studies have examined species trait and environment effects on decomposition by modeling litter as a homogeneous pool with one decomposition rate, techniques are available to evaluate whether multi-pool models (with distinct decay rates) are appropriate (Wieder and Lang 1982). Empirical studies that disentangle species trait effects from environmental effects on the decomposition of multiple litter fractions will evaluate whether the effects of environmental variation differ among fractions that decompose at different rates.

The objective of our study was to examine the effects of environmental conditions, specifically moisture, on litter

decomposition by independently manipulating the direct effects of moisture and plant species trait variation across a hydrologic gradient. We studied 12 closely related species from a single evolutionary lineage (the willow family, Salicaceae) that are part of on-going research in a prairie-pothole ecosystem in Minnesota (Savage and Cavender-Bares 2012). The species in this study occur in a range of habitats across a natural hydrologic gradient, from perennially saturated wetlands to mesic habitats (e.g., prairies and seasonally wet meadows; Table 1). Previous research in this ecosystem has demonstrated that Salicaceae species that occupy contrasting hydrologic niches also differ in their physiology, growth rates, and functional traits (Savage et al. 2009; Savage and Cavender-Bares 2011, 2012). For example, species originating from upland (mesic) habitats tend to have higher drought tolerance (e.g., a lower turgor loss point, higher wood density, and greater photoprotective responses of leaf pigments to drought) and slower relative growth rates compared to wetland species when grown under common garden conditions (Savage et al. 2009; Savage and Cavender-Bares 2011, 2012). Finally, interspecific variation in plant traits among Salicaceae species is larger than intraspecific variation (Savage 2010; Savage and Cavender-Bares 2013). Consequently, Salicaceae is a useful family for exploring how variation in hydrologic conditions influences plant tissue decomposition via environment-trait relationships, in comparison to the direct effects of moisture.

In light of the roles of plant species traits and environmental conditions on plant leaf litter decomposition, we hypothesized that species originating from upland habitats, which have higher drought tolerance and greater allocation to structural compounds like lignin, will decompose more slowly than species originating from wetland habitats (Species trait effects—Hypothesis 1). Second, we hypothesized that leaf litter will decompose at a slower rate in wetland habitats than in upland habitats because anaerobic conditions in wetland habitats will inhibit microbial decomposition relative to upland habitats (Moisture effects—Hypothesis 2). Finally, we hypothesized that the combined effects of species origin and habitat moisture will be offsetting, resulting in no net difference in decomposition when leaves are decomposed in situ (i.e., in their native habitats; Combined effects—Hypothesis 3). The negative effects of species traits on decomposition will counteract the positive effects of more aerobic conditions when upland species decompose in upland habitats. Furthermore, the positive effects of species traits on decomposition will counteract the negative effects of anaerobic conditions when wetland species decompose in wetland habitats. Therefore, we predicted no net difference in decomposition between wetland and upland species in their respective native habitats.

Table 1 Native habitat affinity, growth form, and initial leaf litter chemistry of the 12 species (willow family, Salicaceae) studied

| Species | Habitat affinity | Growth form | SCC | HBP | CELL | LR | N | P | CT |
|--------------------------|------------------|-------------|----------|----------|----------|---------|--------------|-------------|----------|
| <i>Salix lucida</i> | Wetland | Shrub | 553 (12) | 128 (12) | 141 (3) | 174 (2) | 12.16 (0.14) | 1.44 (0.03) | 315 (21) |
| <i>S. pyrifolia</i> | Wetland | Shrub | 613 (7) | 110 (9) | 120 (6) | 155 (2) | 10.21 (0.14) | 1.02 (0.04) | 248 (29) |
| <i>S. nigra</i> | Intermediate | Tree | 452 (9) | 105 (14) | 123 (3) | 317 (4) | 17.34 (0.35) | 1.56 (0.01) | 614 (39) |
| <i>S. petiolaris</i> | Intermediate | Shrub | 617 (7) | 95 (10) | 120 (5) | 165 (3) | 9.27 (0.19) | 1.10 (0.02) | 146 (4) |
| <i>S. amygdaloides</i> | Intermediate | Tree | 567 (13) | 115 (11) | 118 (4) | 197 (1) | 12.53 (0.10) | 1.36 (0.03) | 245 (11) |
| <i>S. bebbiana</i> | Intermediate | Shrub | 591 (8) | 98 (6) | 119 (2) | 187 (4) | 10.80 (0.19) | 2.16 (0.07) | 527 (56) |
| <i>S. discolor</i> | Intermediate | Shrub | 613 (9) | 85 (9) | 116 (2) | 182 (1) | 8.60 (0.16) | 1.60 (0.07) | 327 (18) |
| <i>S. eriocephala</i> | Upland | Shrub | 558 (11) | 108 (12) | 135 (2) | 195 (1) | 13.16 (0.05) | 1.79 (0.02) | 396 (14) |
| <i>Populus deltoides</i> | Upland | Tree | 542 (12) | 153 (12) | 168 (4) | 134 (1) | 13.43 (0.75) | 2.86 (0.08) | 18 (3) |
| <i>P. tremuloides</i> | Upland | Tree | 505 (1) | 126 (8) | 158 (10) | 207 (3) | 10.20 (0.55) | 1.17 (0.05) | 251 (27) |
| <i>S. humilis</i> | Upland | Shrub | 445 (15) | 95 (16) | 191 (2) | 261 (4) | 8.69 (0.12) | 0.72 (0.01) | 126 (22) |
| <i>S. interior</i> | Upland | Shrub | 499 (12) | 78 (17) | 157 (4) | 265 (5) | 14.43 (0.04) | 2.33 (0.06) | 286 (25) |

Species are ordered based on their distribution across a water availability gradient from wet to dry habitats. Growth form and habitat affinity classifications are from field studies of natural populations in Savage and Cavender-Bares (2012). All values are expressed as mg g^{-1} leaf litter and $n = 3$ (standard error in parentheses)

SCC leaf litter initial soluble cell contents, HBP leaf litter initial hemicellulose plus bound proteins, CELL leaf litter initial cellulose, LR leaf litter initial lignin plus recalcitrants, N leaf litter initial nitrogen, P leaf litter initial phosphorus, CT leaf litter initial condensed tannins

Materials and methods

We established a 3-year leaf litter decomposition study at ten sites located in paired wetland–upland habitats throughout Cedar Creek Ecosystem Science Reserve (East Bethel, MN, 45°25'N, 93°10'W; Wei et al., unpublished manuscript). Soils are sandy and are developed from glacial outwash (Grigal and Homann 1994). The water table is shallow and small variation in elevation across short distances causes ecologically meaningful differences in available moisture. At each site, there were two plots, one wetland plot and one upland plot, located adjacent to and upslope from the wetland plot.

Gravimetric soil moisture ($\text{g water g dry soil}^{-1}$) was measured from a sieved composite of three soil cores (0–10 cm deep) collected monthly from each plot during each growing season of the 3-year experiment. We calculated plot-level average growing season gravimetric soil moisture by computing the mean gravimetric soil moisture during the growing season months of the experiment (May–September). Additionally, in 2012, depth to water table was measured monthly at all plots using wells (Wei et al., unpublished data). Wells were installed by burying soil-free 5-cm-diameter PVC pipes in the wetland and upland plots. The wells were 0.6 m deep in wetland plots and 1.5 m deep in upland plots.

During the first 2 years of the experiment, time-integrated plant and microbial available nitrogen (N) was quantified using ion-exchange resin (IER) bags (extracted using 2 M KCl) placed at 10 cm below the soil surface for

two consecutive 2-month intervals during the growing season (4 June through 1 August 2011 and 1 August through 27 September 2011) and one fall–winter–spring interval (27 September 2011 through 8 June 2012). Soil pH was measured monthly during the 2011 growing season using a 1:1 soil:water slurry from homogenized soil samples (0–10 cm).

To make leaf litter decomposition bags, freshly fallen leaf litter was collected during September and October 2010 from natural populations of 12 closely related species in the willow family (Salicaceae; see Table 1 for list of species). Collection populations were selected using data from previous field studies of natural populations in which water availability in the species' natural habitats was characterized (Savage and Cavender-Bares 2012). We collected from populations at Cedar Creek, as well as nearby Carlos Avery State Wildlife Management Area (Forest Lake, MN, 45°19'N, 93°05'W), that were located in habitats representative of the species' water regime affinities.

After air-drying the leaf litter, a subsample was ground in a Wiley Mill (0.425 mm). Initial leaf litter chemistry of the ground subsample was analyzed for: concentrations of carbon (C) and N (COSTECH ESC 4010 Elemental Analyzer, Valencia, CA, USA), phosphorus (P) by digestion with 10 N sulfuric acid after ashing samples at 300 °C for 30 min followed by 500 °C for 2 h (DeMott et al. 1998), C fractions (soluble cell contents, SCC; cellulose, CELL; hemicellulose plus bound proteins, HBP; and lignin plus other recalcitrant compounds, LR; Ankom Fiber Analyzer, Ankom Technology, Macedon, NY, USA), and condensed

tannins (CT) by acid butanol assay with quebracho extract standard (Porter et al. 1986).

Leaf litter decomposition bags (15 cm × 15 cm size) were constructed out of 1-mm fiberglass mesh. Each bag was filled with 3 g air-dried leaf litter. Additional leaf litter was also dried for >48 h at 65 °C to calculate an air-dried:oven-dried weight conversion factor for each species. Replicate bags were constructed to allow for six harvests of each species in each plot (wetland or upland). Litter bags were deployed at the start of winter in 2010 (27 November 2010). Bags were deployed at a minimum of six (of ten) randomly selected sites. Where there was sufficient leaf litter material, bags were deployed at all ten sites.

Leaf litter decomposition bags were harvested from each species–plot–site combination six times over the course of 3 years. Since litter mass loss occurs rapidly during the first year of decomposition, there were four harvests in 2011 (29 April, 26 May, 1 August, 17 September). The remaining harvests were in 2012 (30 September) and 2013 (20 September). At each harvest, litter was removed from the bags and cleaned of debris, including in-grown roots. Clean leaf litter was dried at 65 °C for >48 h in a forced-air drying oven and weighed. We calculated mass loss (% initial mass) and analyzed % C and % N of the remaining litter sample (COSTECH ESC 4010 Elemental Analyzer, Valencia, CA, USA).

In order to evaluate the relationship between litter traits that directly affect decomposition and plant physiology, growth rate, and functional traits that vary across the hydrologic gradient, we supplemented our leaf litter chemistry data with additional green leaf and plant trait data from previous studies. We used green leaf maximum photosynthetic capacity (A_{\max} ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), green leaf specific leaf area (SLA; cm g^{-1}), green leaf turgor loss point (TLP, the leaf water potential at which a species wilts; MPa), and plant wood density (WD; g cm^{-3}) measurements from field studies (Savage 2010; Savage and Cavender-Bares 2012). TLP and WD were selected in particular because previous research showed those traits to be significant indicators of drought tolerance (Savage and Cavender-Bares 2012). Furthermore, we used total plant relative growth rate (RGR; $\text{g g}^{-1} \text{ day}^{-1}$) measurements from a greenhouse study where the species were grown under common conditions where water was non-limiting (Savage and Cavender-Bares 2012). SLA, TLP, and WD trait data were available for all 12 of the species studied here, RGR data were available for 11 species, and A_{\max} data were available for 10 species. Finally, water availability in each species' native habitat (WT, depth to water table in meters during the driest month of the year; Savage and Cavender-Bares 2012) was used as a proxy variable for known (Savage et al. 2009; Savage and Cavender-Bares 2011, 2012) and unknown plant species traits that could covary with native habitat moisture.

Data analysis

All analyses were performed using R (R v.3.0.1; R Foundation for Statistical Computing, Vienna, 2013). The effects of plot type (wetland vs. upland plots) on soil moisture (2011–2013 growing season average gravimetric soil moisture of each plot), depth to water table (2012 growing season average depth to water table at each plot), soil pH, and available N were analyzed using one-way ANOVA.

The effect of species identity on initial leaf litter chemistry was analyzed using one-way ANOVA for each litter variable. Relationships among the plant trait variables, including WT (depth to water table the driest month of the year) in the species' native habitat, were analyzed with pairwise correlations. Significance of the correlation was assessed using a simple linear regression model. Relationships between WT and green leaf and plant traits have been previously reported for 15 species in the Salicaceae family (Savage and Cavender-Bares 2012). Results from correlations between traits for the 12 species included in this study are reported here.

To evaluate the effects of plot and litter type on decomposition rate, we fit our leaf litter decomposition data (i.e., proportion of initial leaf litter mass at each time point) to exponential decay models. First, we selected a model that was the best fit for our data by comparing the fit of the leaf litter decomposition data to three different decomposition models (Wieder and Lang 1982): a single-exponential decomposition model, $X = e^{-kt}$; a double-exponential decomposition model, $X = Ce^{-k_1t} + (1 - C)e^{-k_2t}$; and an asymptotic model, $X = A + (1 - A)e^{-k_a t}$. In all models, X is the proportion of initial mass remaining at time t . In the single-exponential model, k is the decomposition rate. In the double-exponential decomposition model, k_1 is the decomposition rate of the more slowly decomposing fraction of the initial mass (C) and k_2 is the decomposition rate of the more rapidly decomposing fraction of the remaining mass ($1 - C$). Finally, in the asymptotic model, A is the fraction of initial mass that decomposes at a rate of zero (i.e., the asymptotic mass remaining) and k_a is the decomposition rate of the remaining mass ($1 - A$; i.e., the more rapidly decomposing fraction). All models were fit using the `bbmle` package in R. We used the Akaike Information Criterion (AIC) as a measure of model fit.

Since the asymptotic model was the best fit for the data (see “Decomposition models”), we calculated the decomposition rate (k_a) and asymptotic mass remaining (i.e., the proportion mass remaining at which $k = 0$; A) for each substrate-plot (wetland or upland plot) combination at each site. The asymptotic model, like all of the decomposition models we considered, makes simplifying assumptions (Wieder and Lang 1982). First, the model assumes that the modeled pools are discrete. Furthermore, the asymptotic

model assumes that once the more rapidly cycling litter pool decomposes at rate k_a , the remaining pool is completely resistant to decomposition ($k = 0$). Since no litter fraction will have a decay rate of 0, the fact that the asymptotic model was the best fit for our data suggests that the decay rate of the remaining litter pool is very slow. Despite these simplifying assumptions, this modeling approach allowed us to examine how the decay rates and sizes of distinct litter pools (i.e., rapidly versus very slowly cycling litter fractions) varied across litter types and habitats.

We used both of the modeled decomposition variables, k_a and A , as response variables in analyses of the effects of moisture in the decomposition environment, plant species traits, as well as their interaction, on decomposition. In all analyses, gravimetric soil moisture (2011–2013 growing season average) was used as the measurement of moisture in the decomposition environment because this, as opposed to depth to water table, was the most appropriate indicator of water availability for microbial decomposition. We analyzed the effects of plant species traits, including species' native habitat water availability, using multiple linear regressions. Since many plant trait variables were correlated with one another (see "Plant species traits"), each trait variable was analyzed in a separate model with plot moisture and the trait by moisture interaction term. In all analyses the interaction term was removed from the model when non-significant.

Finally, we examined N dynamics during leaf litter decomposition by comparing the proportion of initial N remaining to the proportion of initial mass remaining during the first 2 years of decomposition (2011–2012) for each species (Parton et al. 2007).

Results

Environmental differences between upland and wetland plots

Average growing season gravimetric soil moisture was more than three times higher in the wetland plots than in the upland plots (upland plot average = 0.14 g water g⁻¹ dry soil; wetland plot average = 0.55 g water g⁻¹ dry soil; $F = 68.871$, $df = 1, 18$, $P < 0.001$, $R^2 = 0.781$; see Online Resource, Fig. 1). Furthermore, average depth to water table during the 2012 growing season was more than four times deeper in upland than wetland plots (upland plot average = -0.81 m, wetland plot average = -0.17 m; $F = 57.284$, $df = 1, 18$, $P < 0.001$, $R^2 = 0.748$; see Online Resource, Fig. 1). Soil pH was significantly higher in the upland plots (upland plot average = 5.56, wetland plot average = 5.27; $F = 5.08$, $df = 1, 18$, $P = 0.037$, $R^2 = 0.177$). Soil ammonium availability was significantly

higher in the wetland plots (upland plot average = 0.39 mg NH₄⁺ g⁻¹ resin, wetland plot average = 1.27 mg NH₄⁺ g⁻¹ resin; $F = 1.14$, $df = 1, 18$, $P = 0.016$, $R^2 = 0.254$). Upland and wetland plot soil nitrate did not differ (upland plot average = 0.053 mg NO₃⁻ g⁻¹ resin, wetland plot average = 0.051 mg NO₃⁻ g⁻¹ resin; $F = 0.005$, $df = 1, 18$, $P = 0.94$, $R^2 = 0.000$).

Plant species traits

There was a significant effect of species identity on all initial leaf litter chemistry variables ($df = 11$ and $P < 0.01$ for all ANOVA tests; Table 1). Many pairwise comparisons of initial leaf litter traits were significantly correlated with one another (see Online Resource, Table 1). Specifically, initial leaf litter C constituents (SCC, HBP, CELL, and LR) were highly correlated: initial soluble cell content concentration was negatively correlated with initial cellulose concentration ($r = -0.682$; $t = -2.949$, $df = 10$, $P = 0.015$) and with initial lignin plus recalcitrant concentration ($r = -0.810$; $t = -4.368$, $df = 10$, $P = 0.0014$). There was a weak positive correlation between initial leaf litter N and P concentrations ($r = 0.504$; $t = 1.843$, $df = 10$, $P = 0.095$) and a weak negative correlation between initial leaf litter condensed tannin and cellulose concentrations ($r = -0.532$; $t = -1.982$, $df = 10$, $P = 0.076$).

We did not observe significant relationships between leaf litter chemistry and green leaf traits; however, one plant trait (wood density) was significantly positively correlated with initial leaf litter cellulose concentration ($r = 0.627$; $t = 2.548$, $df = 10$, $P = 0.029$) and significantly negatively correlated with initial leaf litter soluble cell content concentration ($r = -0.593$; $t = -2.327$, $df = 10$, $P = 0.042$). Furthermore, there was a marginally significant negative correlation between wood density and turgor loss point ($r = -0.571$; $t = -2.200$, $df = 10$, $P = 0.053$).

Finally, there were significant (or near significant) correlations between water availability in the species' native habitats (WT) and species' leaf litter chemistry, green leaf, and wood traits, where a positive correlation with WT indicates that the trait value increased as native habitat moisture increased (from more negative WT values in dry habitats to less negative WT values in wet habitats) and a negative correlation with WT indicates that the trait value decreased as native habitat moisture increases. WT was positively correlated with initial soluble cell content concentration ($r = 0.513$; $t = 1.891$, $df = 10$, $P = 0.088$), significantly negatively correlated with initial cellulose concentration ($r = -0.747$; $t = -3.553$, $df = 10$, $P = 0.0052$), significantly positively correlated with turgor loss point ($r = 0.746$; $t = 3.545$, $df = 10$, $P = 0.0053$), and significantly negatively correlated with wood density ($r = -0.592$; $t = -2.323$, $df = 10$, $P = 0.043$).

Decomposition models

After 3 years of decomposition in the field, litter bags lost between 28.2 and 88.2 % of their initial mass (mean = 52.5 %, median = 52.1 %; see Online Resource, Fig. 2). For each substrate and plot type combination (6–10 replicates per combination), the asymptotic decomposition model was the best fit for 70.8 % of the cases (17 out of 24), while the double-exponential decomposition model was the best fit for 29.2 % of the cases (7 out of 24). The single-exponential decomposition model was never the best fit (0 out of 24; difference in AIC between the single-exponential model and the other two models was always ≥ 5). In all of the cases, the difference in AIC between the double-exponential and asymptotic models was < 1 , which indicates that the asymptotic and double-exponential models are indistinguishable from each other. Furthermore, the decay rate of the slower cycling pool in the double-exponential model was 0 in 95.8 % of the cases (23 out of 24), i.e., the double exponential model collapsed to an asymptotic model.

Therefore, we calculated the decomposition rate (k_a) of the quickly decomposing litter fraction and asymptotic mass remaining (A) for each substrate–plot–site combination. We excluded one substrate–plot–site combination that had an extremely poor model fit ($R^2 = 0$), due to excessive sand adhering to the litter at several collection points. The asymptotic model fits (R^2) of the remaining data ranged from 0.39–0.99, with a mean $R^2 = 0.85$ and a median $R^2 = 0.90$ [see Online Resource, Table 2 for average model fit (R^2), k_a and A parameter estimates for each substrate–plot combination].

Effects of plant species traits and moisture on decomposition rate (k_a)

Several plant species leaf litter traits significantly influenced decomposition rates (k_a) of the quickly decomposing litter fraction (see Online Resource, Table 3). Specifically, initial litter soluble cell content concentration was positively related to k_a ($t = 4.552$, $df = 189$, $P < 0.001$, $R^2 = 0.091$) and initial litter cellulose concentration and initial litter lignin plus recalcitrant concentration were negatively related to k_a (CELL: $t = -4.778$, $df = 189$, $P < 0.001$, $R^2 = 0.100$; LR: $t = -3.427$, $df = 189$, $P < 0.001$, $R^2 = 0.050$). Finally, there was a significant interaction between moisture in the decomposition environment and initial litter hemicellulose plus bound protein concentration ($t = 2.512$, $df = 188$, $P = 0.013$, $R^2 = 0.030$): hemicellulose plus bound protein concentration increased k_a in the wetland plots and decreased decomposition rate in the upland plots.

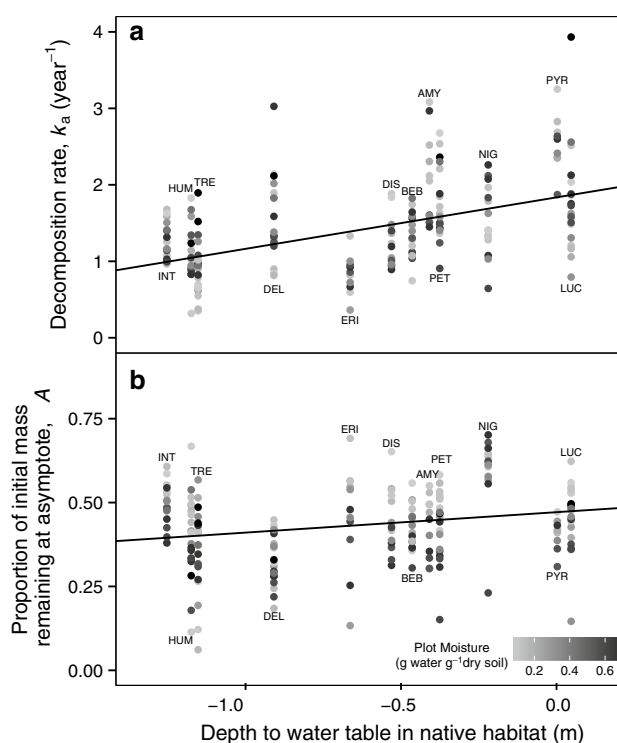


Fig. 1 Effects of plot moisture and moisture in the species' native habitat on **a** decomposition rate and **b** asymptotic mass remaining. **a** The moisture in the species' native habitat (WT), but not plot moisture, significantly influenced the decomposition rate (k_a) of leaf litter (main effect WT, $P < 0.001$; main effect plot moisture, $P = 0.45$; model $R^2 = 0.240$). **b** The moisture in the species' native habitat, as well as plot moisture, significantly influenced the asymptotic mass remaining (A) of leaf litter (main effect WT, $P < 0.001$; main effect plot moisture, $P < 0.001$; model $R^2 = 0.132$). The darkness of each symbol shows plot moisture (g water g^{-1} dry soil). Species identity is represented by three-letter codes: AMY *S. amygdaloides*, BEB *S. bebbiana*, DEL *P. deltoides*, DIS *S. discolor*, ERI *S. eriocephala*, HUM *S. humilis*, INT *S. interior*, LUC *S. lucida*, NIG *S. nigra*, PET *S. petiolaris*, PYR *S. pyrifolia*, TRE *P. tremuloides*

In addition to leaf litter traits, several green leaf and plant traits were related to k_a . Turgor loss point and specific leaf area were significantly positively related to k_a (TLP: $t = 5.389$, $df = 189$, $P < 0.001$, $R^2 = 0.125$; SLA: $t = 2.708$, $df = 189$, $P = 0.007$, $R^2 = 0.029$), while wood density was significantly negatively related to k_a ($t = -7.159$, $df = 189$, $P < 0.001$, $R^2 = 0.206$). There was a significant positive relationship between plant species relative growth rate and k_a ($t = 3.536$, $df = 172$, $P < 0.001$, $R^2 = 0.060$).

Moisture availability in the species' native habitat was significantly positively related to k_a ($t = 7.865$, $df = 189$, $P < 0.001$, $R^2 = 0.240$): species originating from drier habitats decomposed more slowly than species originating from wetter habitats (Fig. 1a). Although native habitat water availability is not a plant trait itself, it is representative of

plant traits that vary along the hydrologic gradient that may influence decomposition.

There was not a consistent effect of moisture in the decomposition habitat on k_a : some species decomposed faster while others decomposed slower in the wetland plots (main effect plot moisture $P = \text{n.s.}$ for most models; see Online Resource, Table 3).

Effects of plant species traits and moisture on asymptotic mass remaining (A)

As with decomposition rate (k_a), several species' leaf litter chemistry traits influenced A (see Online Resource, Table 4). There were significant positive relationships between A and leaf litter initial N concentration ($t = 5.086$, $df = 189$, $P < 0.001$, $R^2 = 0.194$), initial condensed tannin concentration ($t = 6.647$, $df = 189$, $P < 0.001$, $R^2 = 0.258$), and initial lignin plus recalcitrant concentration ($t = 5.896$, $df = 189$, $P < 0.001$, $R^2 = 0.226$). There was a significant negative relationship between A and initial cellulose concentration ($t = -3.578$, $df = 189$, $P < 0.001$, $R^2 = 0.142$) and a marginally significant negative relationship between A and soluble cell contents ($t = -1.815$, $df = 189$, $P = 0.071$, $R^2 = 0.100$). There was a significant interactive effect of initial hemicellulose plus bound protein concentration and moisture in the decomposition environment on A ($t = 2.087$, $df = 188$, $P = 0.038$, $R^2 = 0.186$): A decreased with increasing concentration of hemicellulose plus bound proteins in both habitats; however, the slope of the effect was greater (more negative) in the upland plots compared to the wetland plots.

Green leaf and plant traits also influenced A . Turgor loss point and A_{max} were significantly positively related to A (TLP: $t = 5.260$, $df = 189$, $P < 0.001$, $R^2 = 0.201$; A_{max} : $t = 2.701$, $df = 160$, $P = 0.0077$, $R^2 = 0.181$). There was a marginally significant interactive effect of green leaf specific leaf area and moisture in the decomposition environment on A ($t = 1.711$, $df = 188$, $P = 0.089$, $R^2 = 0.094$): A decreased with green leaf specific leaf area in the upland plots and increased in the wetland plots. Species relative growth rate was significantly positively related to A ($t = 4.758$, $df = 189$, $P < 0.001$, $R^2 = 0.185$), while wood density was significantly negatively related to A ($t = -2.096$, $df = 189$, $P = 0.038$, $R^2 = 0.105$).

Water availability in the species' native habitat (WT) was significantly related to A . Species that originated from wetter habitats had more asymptotic mass remaining than species originating from drier habitats (main effect WT: $t = 3.234$, $df = 189$, $P = 0.0014$; $R^2 = 0.132$; Fig. 1b). However, the models that included WT, or other traits that covary with WT, had relatively poor model fits (low R^2). Instead, models that included traits that did not covary with moisture in the species' native habitat (i.e., litter condensed

tannins and lignin plus recalcitrants) had the best model fits (highest R^2 ; see Fig. 2 and Online Resource, Table 4).

Finally, moisture in the decomposition environment significantly decreased A . In general, A was higher in drier plots than in wetter plots, with the exception of a few species (i.e., *Populus tremuloides* and *Salix nigra*; see Fig. 1b).

Litter nitrogen dynamics

All but two of the species (*S. interior* and *S. nigra*) exhibited microbial net N immobilization during the first 2 years of decomposition (Fig. 3). Net N immobilization was greatest for the species with the lowest initial N concentration and decreased with increasing initial N. Litter N dynamics did not differ with moisture in the decomposition environment (wetland versus upland plots; see Online Resource, Fig. 3).

Discussion

Combined effects of moisture and species traits on decomposition

We found that the quickly and slowly cycling litter fractions had contrasting responses to the combined effects of moisture and species traits. For the quickly cycling litter fraction, species traits associated with habitat origin affected litter decomposition: the quickly cycling litter fraction of wetland habitat species—which had a higher concentration of more labile, easier to degrade biomolecules (i.e., soluble cell contents)—decomposed faster than the quickly cycling litter fraction of upland habitat species. Furthermore, moisture of the decomposition habitat did not affect decomposition rate of this fraction. Thus, when decomposed in their respective native habitats, the quickly cycling litter fraction of wetland species tended to decompose faster than the quickly cycling litter fraction of upland species, although overall the models had little predictive power (low R^2). This trend towards greater decomposition of wetland species relative to upland species when decomposed in situ is in contrast to our Hypothesis 3, which predicted no net difference in decomposition when leaf litter is decomposed in its native habitat. However, the effect is consistent with a meta-analysis by Cornwell et al. (2008): they suggested that species trait effects lead to greater variation in decomposition than direct environmental effects.

The combined effects of moisture and species traits on decomposition of the slowly cycling litter fraction were less straightforward. In support of Hypothesis 3, moisture and species traits had contrary, as opposed to reinforcing, effects on asymptotic mass remaining. Moisture decreased the mass remaining at asymptote (A) and moisture in the

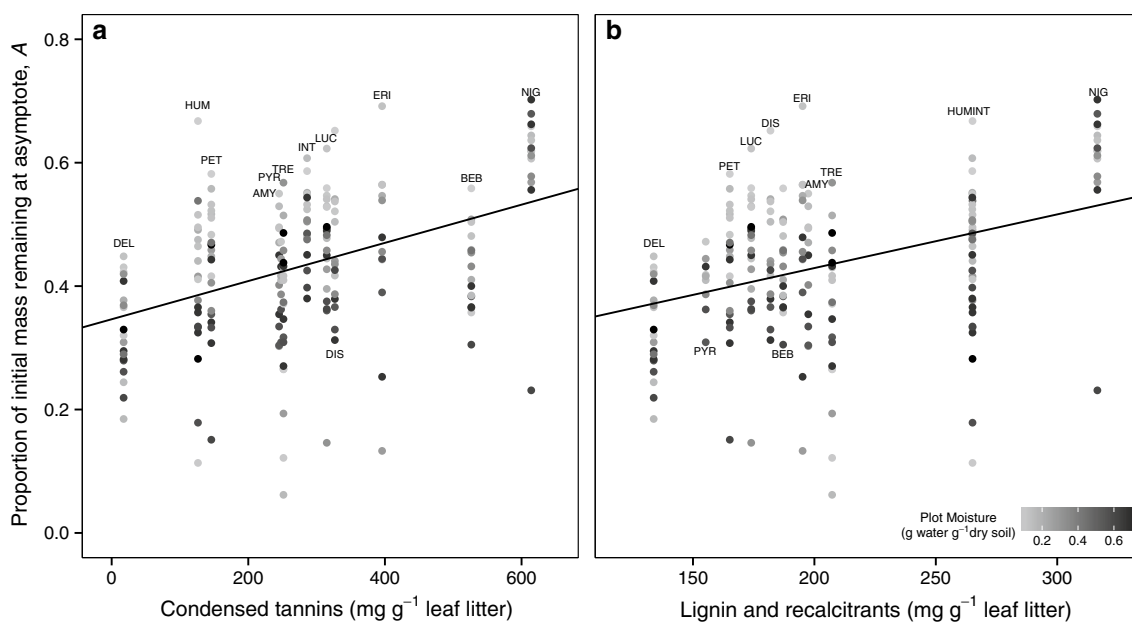


Fig. 2 Effects of leaf litter chemistry on asymptotic mass remaining. **a** Initial leaf litter condensed tannin concentration significantly increased the asymptotic mass remaining (A) of leaf litter, while plot moisture (wetter plots) significantly decreased A (main effect litter condensed tannins, $P < 0.001$; main effect plot moisture, $P < 0.001$; model $R^2 = 0.258$). **b** Initial concentration of leaf litter lignin plus recalcitrants significantly increased the asymptotic mass remaining (A), while plot moisture (wetter plots) significantly decreased A (main

effect litter lignin plus recalcitrants, $P < 0.001$; main effect plot moisture, $P < 0.001$; model $R^2 = 0.226$). The darkness of each symbol shows plot moisture (g water g^{-1} dry soil). Species identity is represented by three-letter codes: AMY *S. amygdaloides*, BEB *S. bebiana*, DEL *P. deltooides*, DIS *S. discolor*, ERI *S. eriocephala*, HUM *S. humilis*, INT *S. interior*, LUC *S. lucida*, NIG *S. nigra*, PET *S. petiolaris*, PYR *S. pyrifolia*, TRE *P. tremuloides*

species' native habitat tended to increase A . However, species' origin along the hydrologic gradient (and traits related to species' origin) was not the best predictor of A (low model R^2). Instead, species traits unrelated to species' origin (e.g., litter condensed tannin and lignin plus recalcitrant concentrations) influenced A . We discuss these species and environmental effects on decomposition in more detail below.

It is not surprising that the quickly and slowly cycling litter fractions showed contrasting responses to species trait and moisture effects. It is well known that litter decomposition dynamics differ among litter fractions (Berg and McClaugherty 2007). Relatively few litter decomposition studies have examined multiple litter fractions (Adair et al. 2010); however, results from these studies suggest that the factors that influence decomposition of each fraction may differ and that the same factor can have contrasting effects among fractions. For example, work by Berg (2000) and Hobbie et al. (2012) has shown that N addition has opposite effects on the rapidly and slowly cycling litter fractions: N increases the decomposition rate of the rapidly cycling litter fraction and decreases the decomposition rate of the more slowly cycling fraction, leading to greater A values.

Species trait effects on decomposition

Based upon theoretical and empirical work on the relationships between the environment, plant species traits, and decomposition, we hypothesized (Hypothesis 1) that plant species originating from upland habitats—which have higher drought tolerance and more structural compounds—would decompose slower than species originating from wetland habitats. In support of our hypothesis, traits associated with species' origin along a hydrologic gradient influenced the decomposition rate of the quickly cycling fraction (k_a): k_a increased with moisture availability in the species' native habitat. Furthermore, the effects of species traits that covaried with species origin (e.g., turgor loss point, wood density, litter soluble cell contents, and litter cellulose) were significant for the decomposition of the rapidly cycling litter fraction. For example, low values of soluble cell contents (SCC) correlated with slower decomposition rates, as well as upland habitat origin; high values of SCC correlated with faster decomposition rates, as well as wetland habitat origin. This suggests that decomposition rate (k_a) may be influenced by species traits that vary across the hydrologic gradient.

In contrast, the results from the slowly cycling litter fraction did not clearly support our hypothesis. Despite

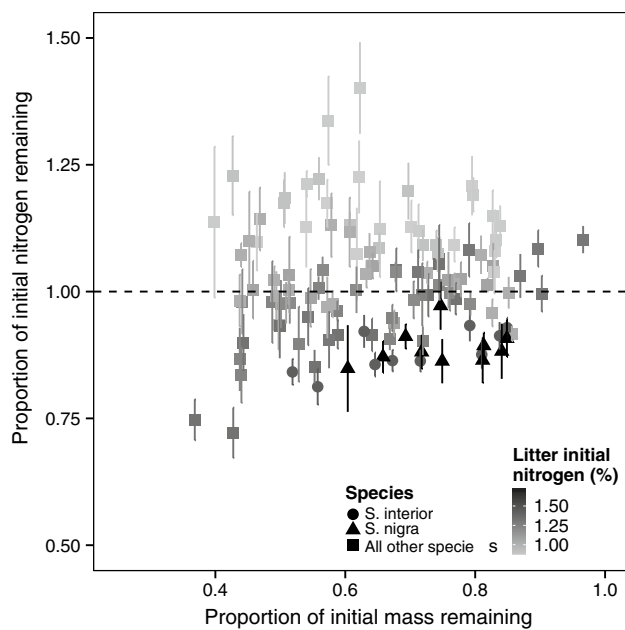


Fig. 3 Nitrogen (N) dynamics during decomposition. Each *symbol* represents replicate litter bags containing leaf material of a single species from a single harvest and habitat collected during the first 2 years of decomposition (5 harvests total). The darkness of each symbol shows initial litter N concentration of the species (expressed as initial percent N). Mean values of proportion of initial N remaining and proportion of initial mass remaining are displayed; *lines* are \pm standard error of the mean proportion of initial N remaining. The *area above the dashed line* indicates net N immobilization: litter N content at harvest exceeds initial litter N content. The *area below the dashed line* indicates net N mineralization: litter N content at harvest is less than initial litter N content. N was immobilized during the first 2 years of decomposition for all species except for *Salix interior* and *S. nigra* (these species, indicated with *round* and *square* symbols, respectively, also had the highest initial N concentrations)

the statistical significance of species' native habitat water table (WT) on A , the R^2 of the model that included WT was relatively low and was likely driven by two species with particularly low and particularly high A values (*P. deltoides* and *S. nigra*, respectively). Instead, the best models of asymptotic mass remaining included plant trait variables that do not covary with water availability in the species' native habitat: initial concentrations of leaf litter condensed tannins and lignin plus recalcitrants. Although we expected (and found) that structural traits, such as condensed tannins and lignin, would influence the decomposition of the slowly cycling fraction, we did not find that these traits also related to habitat origin. This suggests that the decomposition of the slowly cycling fraction, unlike the rapidly decomposing fraction, is influenced by species' traits that vary independently of the hydrologic gradient and are determined by other factors. For example, other work has shown that condensed tannin concentration is influenced by additional factors such

as phylogeny and herbivore pressure (Schweitzer et al. 2008).

The effects of leaf litter C chemistry, such as soluble cell contents, cellulose, and lignin plus recalcitrants, on decomposition are well established (e.g., Cornwell et al. 2008). These traits are the proximate factors to influence decomposition. However, how these tissue traits relate to whole-plant traits (e.g., wood density) or plant physiological traits (e.g., turgor loss point) that vary across the soil moisture gradient that characterizes the species' native environments (and also have significant relationships with decomposition) is less well understood. Although many studies have linked turgor loss point and wood density to other measures of plant physiology (e.g., Bucci et al. 2004), few have analyzed relationships between these traits and leaf traits that impact decomposition. Such studies will better connect plant tissue chemistry traits relevant to decomposition with the plant physiological traits associated with plant trade-offs across soil moisture gradients. Furthermore, it was surprising that some statistically significant litter chemistry variables (i.e., soluble cell contents and cellulose) correlated with species' origin, while others (i.e., lignin plus recalcitrants) did not. Further efforts examining the relationship between plant tissue chemistry and plant physiological trade-offs in more detail may help explain these relationships.

The effects of leaf litter herbivore defense chemistry (i.e., concentration of condensed tannins) on decomposition are similarly well established (Cornelissen et al. 2004), especially among species in the Salicaceae family (Schweitzer et al. 2008). Numerous studies have reported negative effects of initial condensed tannin concentration on decomposition rate (k) using a single-exponential decomposition model (Driebe and Whitham 2000; Schweitzer et al. 2004; LeRoy et al. 2006). Unfortunately, none of these studies report double-exponential or asymptotic models of leaf litter decomposition. Interestingly, when we fit our data to a single-exponential decomposition model (for the purposes of comparison only since this model was the worst fit), we found a significant negative effect of initial leaf litter condensed tannin concentration on decomposition rate (k) of the single litter pool ($t = -3.041$, $df = 194$, $P = 0.003$, $R^2 = 0.407$). This suggests that our results—the lack of an effect of condensed tannins concentration on decomposition rate (k_a), but an effect for the asymptotic mass remaining (A)—may be consistent with previous work which did not distinguish between multiple litter pools.

Species trait effects on N dynamics

Unlike the decomposition rate of litter (i.e., mass loss), nutrient availability during decomposition appears to be

unrelated to traits associated with species' native habitat position along the hydrologic gradient. Instead, we found that microbial net N immobilization was greatest for those species with low initial N concentration and negligible or absent for species with high initial N concentration. This result is congruent with established global scale patterns of N dynamics during leaf litter decomposition (Parton et al. 2007). Furthermore, leaf litter initial N concentration was unrelated to moisture in the species' native habitat. For example, the two species that exhibited no net N immobilization during the first 2 years of decomposition (*S. nigra* and *S. interior*) occur in intermediate and upland habitats, respectively. Consequently, while species traits associated with species' origin will influence decomposition rate (mass loss) of the rapidly cycling litter fraction, N dynamics during decomposition will be affected by the factors that influence species tissue N concentration (e.g., herbivore defense, physiological trade-offs, soil N content; Aerts and Chapin III 2000).

Moisture effects on decomposition

In Hypothesis 2, we expected decomposition to decrease in the wet environment (and, therefore, increase the asymptotic mass remaining) due to anaerobic conditions in wetland habitats (Schoor 2001; Clarkson et al. 2014). Contrary to this hypothesis, we observed no consistent effect of moisture on decomposition rate (k_a), and the size of the slowly decomposing fraction (A) decreased in wetland plots compared to upland plots. Given the plot moisture realized during the experiment, it is unsurprising that moisture did not affect decomposition rate as predicted by our hypothesis. Our wetland habitat plots were rarely surface-saturated (see wetland plot depth to water table in Online Resource, Fig. 1). Consequently, it is likely that aerobic conditions were maintained in the litter bags, which were placed on the soil surface. Since the wettest sites we measured were not permanently saturated, we caution against extending our results to saturated wetland habitats where soil and surface water may inhibit decomposition.

In contrast to our hypothesis, asymptotic mass remaining decreased in wetland plots relative to upland plots. An alternate explanation for decreasing asymptotic mass remaining with increased plot moisture is that higher moisture may have increased leaching and particulate mass loss from the litter bags. Our methodology did not solely track loss via decomposition; instead, soluble material that was leached or particulate material that was washed away was also counted as mass lost (Boulton and Boon 1991). Consequently, this finding may reflect moisture effects on the physical disintegration of litter, as opposed to microbial decomposition.

The effects of moisture on decomposition cannot be completely separated from the effects of pH and nutrient availability on decomposition in this study, since wetland plots also had significantly lower soil pH and higher concentration of ammonium relative to upland plots. pH is a strong predictor of microbial community composition (Fierer and Jackson 2006) and can significantly influence microbial activity. For example, in a decomposition study in aquatic habitats, Chamier (1987) found that microbial decomposition rate (k) decreased with lower pH, possibly due to low microbial colonization of the decomposition substrates under more acidic conditions. Like pH, N availability can also significantly affect the decomposition of multiple litter fractions. For example, Berg (2000) and Hobbie et al. (2012) have found that, in general, higher initial N increases the initial decomposition rate of leaf litter (k_a) and increases the size of the asymptotic mass remaining (A). In contrast to these studies, we found that the effect of moisture on decomposition was not consistent with the effect of pH or N on decomposition reported previously. Specifically, we found that habitat type (wetland or upland) had no effect on the decomposition rate of the fast cycling litter fraction (k_a). Additionally, asymptotic mass remaining (A) was smaller in wetland plots relative to upland plots. Consequently, the observed effects of habitat type on decomposition are consistent with moisture effects on decomposition and not the effect of pH or N availability on decomposition.

Conclusions

Taken together, our results suggest that across a hydrologic gradient spanning upland mesic and wetland (but not permanently saturated) plots, plant traits and moisture have distinct effects on the decomposition of quickly versus slowly cycling litter fractions. Overall, more leaf litter tissue will decompose in wetter habitats because species traits in those habitats promote decomposition of the fast cycling pool of litter, and because higher moisture in those habitats directly reduces the proportion of litter in the slow cycling fraction, i.e., the asymptotic mass remaining (A). However, additional plant litter tissue traits that do not covary with moisture will also influence asymptotic mass remaining, as well as N release during the first 2 years of decomposition. Consequently, while environmental controls on plant tissue chemistry can be used to inform general patterns of mass loss from the rapidly decomposing fraction across a hydrologic gradient, influential plant traits that are not related to moisture availability in the species' native habitat (e.g., concentration of litter lignin plus recalcitrants and condensed tannins) influence asymptotic mass remaining and thus will also need to be considered.

Author contribution statement SEH, JCB, JAS, and XW conceived and designed the experiments. CER, JAS, and XW performed the experiments. CER analyzed the data and wrote the manuscript; other authors provided editorial advice.

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Conflict of interest The authors declare that they have no conflict of interest.

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Electronic Supplementary Material

Contrasting effects of plant species traits and moisture on the decomposition of multiple litter fractions

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Figure Legend

Online Resource, Fig. 1 Moisture differences between upland and wetland plots. Gravimetric soil moisture data are from this study. Well data (depth to water table) are from Wei et al. *unpublished data*. **Panel a** Average gravimetric soil moisture (g water g⁻¹ dry soil) during the 2011-2013 growing seasons (May-Sept.). Uplands plots have significantly lower average gravimetric soil moisture than wetland plots ($F = 68.871$, $df = 1, 18$, $P < .001$). **Panel b** Average depth to water table (m) during the 2012 growing season (May-Sept.). Upland plots have significantly deeper average depth to water table than wetland plots ($F = 57.284$, $df = 1, 18$, $P < .001$)

Online Resource, Table 1 Pearson correlation coefficients (r) from pairwise comparisons among plant trait measurements

| | SCC | HBP | CELL | LR | N | P | CT | SLA | A_{max} | TLP | RGR | WD | WT |
|------------------------|------------|------------|---------------|---------------|----------|---------------------------|---------------|------------|------------------------|------------|------------|---------------|---------------------------|
| | n=12 | n=12 | n=12 | n=12 | n=12 | n=12 | n=12 | n=12 | n=10 | n=12 | n=11 | n=12 | n=12 |
| SCC | 1.0 | -0.029 | -0.682 | -0.810 | -0.456 | 0.044 | -0.098 | 0.019 | -0.325 | 0.221 | -0.302 | -0.593 | 0.513 [†] |
| | | | ** | *** | | | | | | | | * | |
| HBP | | 1.0 | 0.234 | -0.468 | 0.193 | 0.238 | -0.343 | 0.443 | -0.469 | 0.14 | 0.060 | 0.020 | 0.117 |
| CELL | | | 1.0 | 0.224 | -0.049 | 0.056 | -0.531 | -0.380 | 0.326 | -0.460 | -0.195 | 0.627 | -0.747 |
| | | | | | | | † | | | | | * | ** |
| LR | | | | 1.0 | 0.470 | -0.170 | 0.485 | -0.020 | 0.401 | -0.044 | 0.428 | 0.367 | -0.286 |
| N | | | | | 1.0 | 0.504 [†] | 0.444 | 0.400 | 0.221 | 0.451 | 0.443 | -0.084 | 0.076 |
| P | | | | | | 1.0 | 0.053 | 0.163 | 0.168 | 0.129 | -0.325 | -0.037 | -0.228 |
| CT | | | | | | | 1.0 | 0.204 | -0.192 | 0.399 | 0.309 | -0.060 | 0.372 |
| SLA | | | | | | | | 1.0 | -0.444 | -0.087 | 0.210 | -0.181 | 0.261 |
| A_{max} | | | | | | | | | 1.0 | 0.010 | 0.188 | -0.052 | -0.480 |
| RGR | | | | | | | | | | 1.0 | 0.474 | -0.255 | 0.466 |
| TLP | | | | | | | | | | | 1.0 | -0.571 | 0.746 |

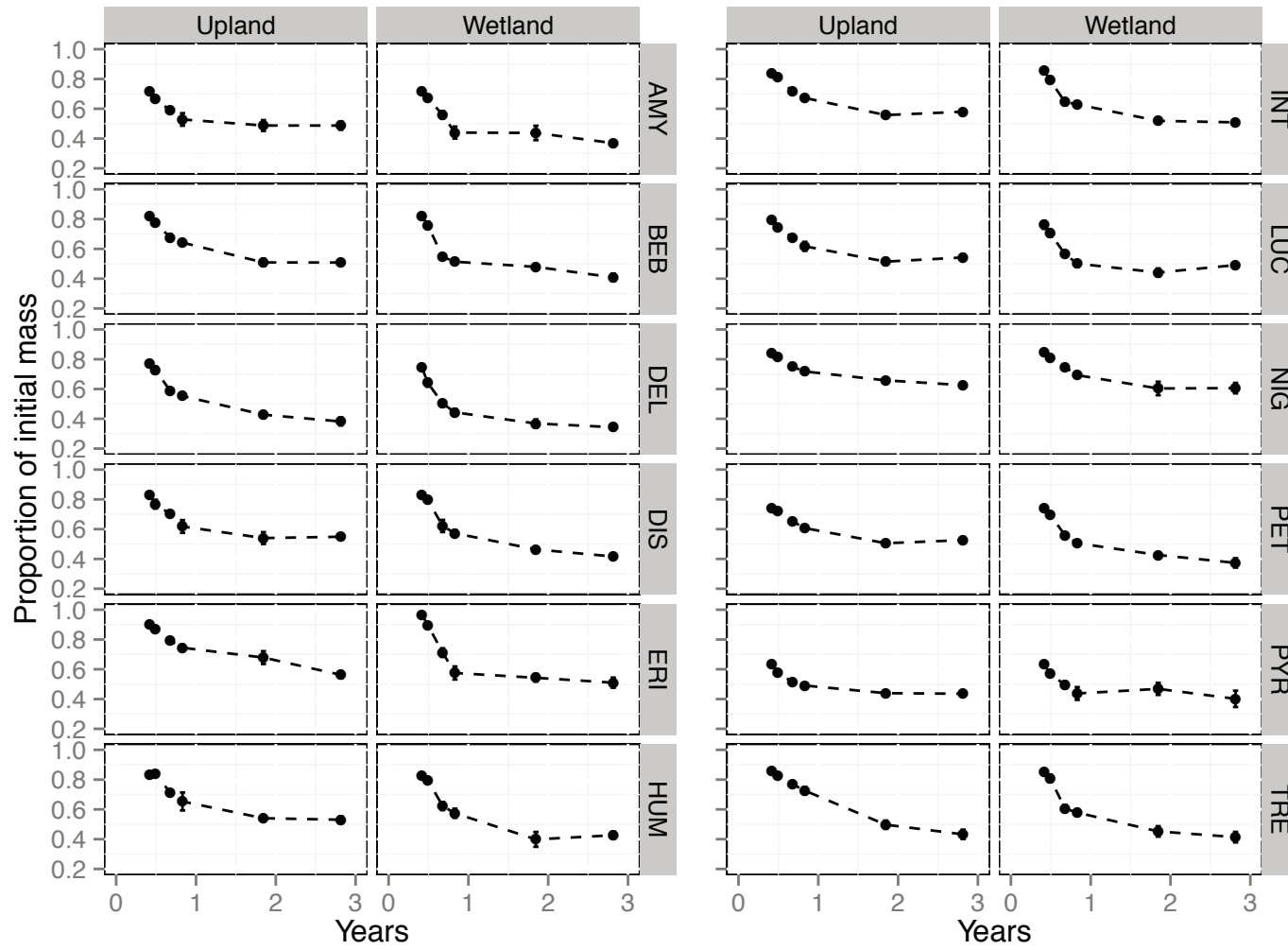
| | | |
|-----------|-----|---------------|
| | † | ** |
| WD | 1.0 | -0.592 |
| | | * |
| WT | | 1.0 |

Leaf litter chemistry trait data is from this study. Green leaf trait data, plant trait data, and depth to water table in the species' native habitat is from Savage and Cavender-Bares (2012). Key to abbreviations: SCC, leaf litter initial soluble cell contents; HBP, leaf litter initial hemicellulose plus bound proteins; CELL, leaf litter initial cellulose; LR, leaf litter initial lignin plus recalcitrants; N, leaf litter initial nitrogen; P, leaf litter initial phosphorus; CT, leaf litter initial condensed tannins; SLA, green leaf specific leaf area; A_{max} , green leaf maximum photosynthetic capacity; TLP, turgor loss point; RGR, plant relative growth rate; WD, wood density; WT, depth to water table in the species' native habitat. n refers to the number of species included in the correlation.

† $P \leq .1$; * $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$

Figure Legend

Online Resource, Fig. 2 Litter bag mass loss over time. Each symbol represents replicate litter bags containing leaf material of a single species from a single habitat (upland or wetland habitat). Mean values of proportion of initial mass remaining are displayed; lines are +/- standard error of the mean proportion of initial mass remaining. Species identity is represented by three-letter codes: AMY=*S. amygdaloides*, BEB=*S. bebbiana*, DEL=*P. deltoides*, DIS=*S. discolor*, ERI=*S. eriocephala*, HUM=*S. humilis*, INT=*S. interior*, LUC=*S. lucida*, NIG=*S. nigra*, PET=*S. petiolaris*, PYR=*S. pyrifolia*, TRE=*P. tremuloides*



Online Resource, Fig. 2

Online Resource, Table 2 Parameter estimates from asymptotic decomposition models.

| Species | Upland plots | | | Wetland plots | | |
|---------------------------|--------------|--------|------------------------|---------------|--------|------------------------|
| | k_a | A | Model fit (R^2) | k_a | A | Model fit (R^2) |
| | 2.27 | 0.47 | 0.88 | 1.82 | 0.37 | 0.79 |
| <i>Salix amygdaloides</i> | (0.21) | (0.04) | (0.04) | (0.29) | (0.03) | (0.10) |
| | 1.24 | 0.46 | 0.91 | 1.43 | 0.40 | 0.78 |
| <i>S. bebbiana</i> | (0.10) | (0.02) | (0.03) | (0.10) | (0.02) | (0.04) |
| | 1.36 | 0.34 | 0.88 | 1.71 | 0.32 | 0.83 |
| <i>Populus deltoides</i> | (0.16) | (0.03) | (0.03) | (0.18) | (0.02) | (0.05) |
| | 1.46 | 0.52 | 0.84 | 1.12 | 0.38 | 0.85 |
| <i>S. discolor</i> | (0.12) | (0.03) | (0.06) | (0.09) | (0.02) | (0.07) |
| | 0.81 | 0.49 | 0.81 | 0.85 | 0.43 | 0.72 |
| <i>S. eriocephala</i> | (0.13) | (0.08) | (0.09) | (0.05) | (0.04) | (0.06) |
| | 1.11 | 0.45 | 0.86 | 1.08 | 0.34 | 0.87 |
| <i>S. humilis</i> | (0.13) | (0.04) | (0.06) | (0.10) | (0.03) | (0.04) |
| | 1.33 | 0.54 | 0.90 | 1.22 | 0.46 | 0.86 |
| <i>S. interior</i> | (0.10) | (0.02) | (0.02) | (0.06) | (0.02) | (0.04) |
| | 1.66 | 0.51 | 0.86 | 1.92 | 0.41 | 0.75 |
| <i>S. lucida</i> | (0.15) | (0.02) | (0.04) | (0.27) | (0.03) | (0.04) |
| | 1.50 | 0.62 | 0.92 | 1.58 | 0.58 | 0.81 |
| <i>S. nigra</i> | (0.10) | (0.01) | (0.02) | (0.21) | (0.05) | (0.04) |

| | | | | | | |
|-----------------------|--------|--------|--------|--------|--------|--------|
| | 1.86 | 0.50 | 0.87 | 1.61 | 0.36 | 0.91 |
| <i>S. petiolaris</i> | (0.14) | (0.02) | (0.05) | (0.14) | (0.03) | (0.02) |
| | 2.70 | 0.43 | 0.94 | 2.38 | 0.37 | 0.77 |
| <i>S. pyrifolia</i> | (0.17) | (0.01) | (0.01) | (0.18) | (0.03) | (0.05) |
| | 0.66 | 0.30 | 0.92 | 1.18 | 0.40 | 0.81 |
| <i>P. tremuloides</i> | (0.07) | (0.05) | (0.01) | (0.11) | (0.03) | (0.04) |

Values are means (standard errors in parentheses)

Online Resource, Table 3 Results of multiple linear regression models of decomposition rate (k_d) with plant traits, moisture in decomposition environment, and their interaction as explanatory variables

| Plant trait in analysis | Effect | | | R ² of model |
|-------------------------|--------------------------|---|-------|-------------------------|
| | Plant trait variable (T) | Moisture in the decomposition environment (M) | T*M | |
| SCC | *** (+) | NS | NA | 0.091 |
| HBP | NS | * (-) | * (+) | 0.030 |
| CELL | *** (-) | NS | NA | 0.100 |
| LR | *** (-) | NS | NA | 0.050 |
| N | NS | NS | NA | 0.000 |
| P | NS | NS | NA | 0.000 |
| CT | NS | NS | NA | 0.000 |
| SLA | ** (+) | NS | NA | 0.029 |
| A _{max} | NS | NS | NA | 0.000 |
| TLP | *** (+) | NS | NA | 0.125 |
| RGR | *** (+) | NS | NA | 0.060 |
| WD | *** (-) | NS | NA | 0.206 |
| WT | *** (+) | NS | NA | 0.240 |

Each plant trait variable was analyzed in a separate regression model. The sign of the relationship between the variable and decomposition rate (k_d) is indicated in parentheses. Key to abbreviations: leaf litter, green leaf, and plant traits same as Online Resource, Table 1.

[†] $P \leq .1$; * $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$; NS $P > .1$; NA: non-significant ($P > .1$) and interaction term removed from model

Online Resource, Table 4 Results of multiple linear regression models of asymptotic mass remaining (A) with plant traits, moisture in decomposition environment, and their interaction as explanatory variables

| Plant trait in analysis | Effect | | | R ² of model |
|-------------------------|--------------------------|---|-------|-------------------------|
| | Plant trait variable (T) | Moisture in the decomposition environment (M) | T*M | |
| SCC | † (-) | *** (-) | NA | 0.100 |
| HBP | *** (-) | ** (-) | * (+) | 0.186 |
| CELL | *** (-) | *** (-) | NA | 0.142 |
| LR | *** (+) | *** (-) | NA | 0.226 |
| N | *** (+) | *** (-) | NA | 0.194 |
| P | NS | ***(-) | NA | 0.085 |
| CT | *** (+) | *** (-) | NA | 0.258 |
| SLA | NS | * (-) | † (+) | 0.094 |
| A _{max} | ** (+) | *** (-) | NA | 0.181 |
| TLP | *** (+) | *** (-) | NA | 0.201 |
| RGR | *** (+) | *** (-) | NA | 0.185 |
| WD | * (-) | *** (-) | NA | 0.105 |
| WT | ** (+) | ** (-) | NA | 0.132 |

Each plant trait variable was analyzed in a separate regression model. The sign of the relationship between the variable and asymptotic mass remaining (A) is indicated in parentheses.

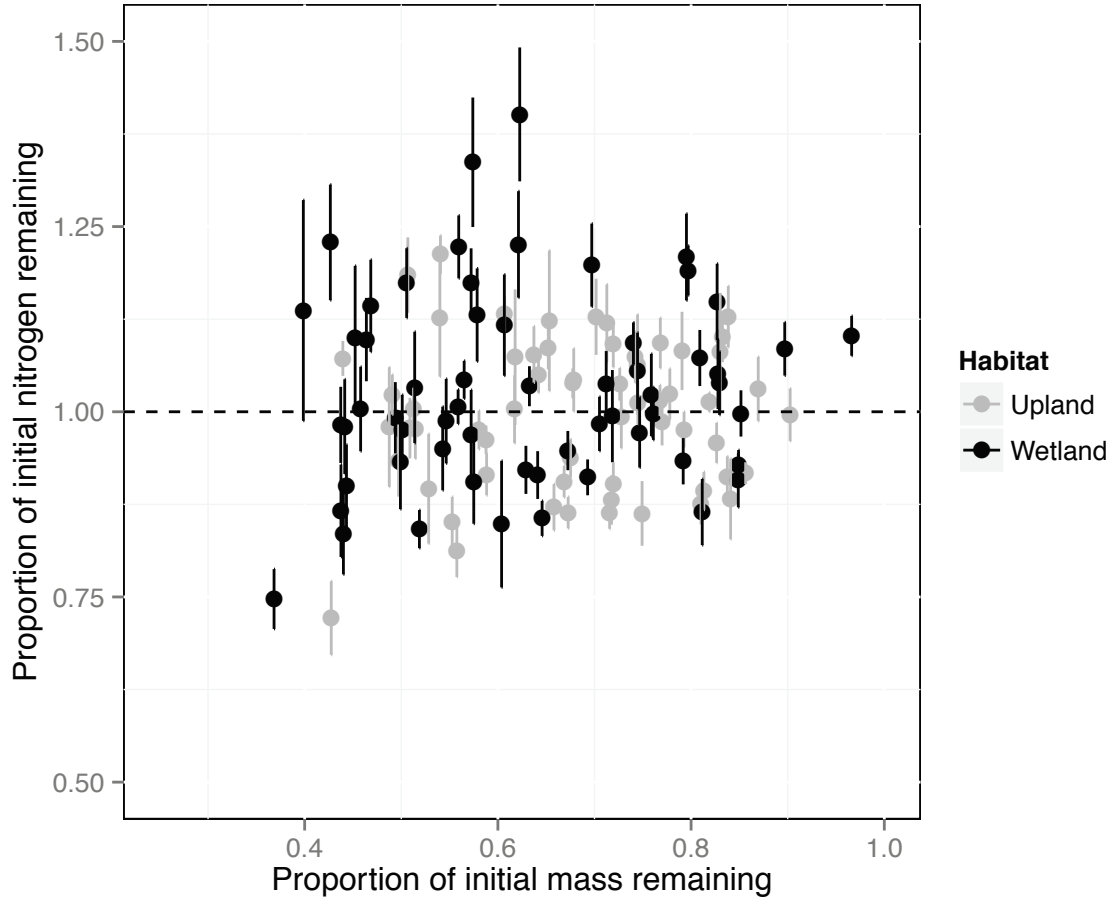
Key to abbreviations: leaf litter, green leaf, and plant traits same as Online Resource, Table 1.

† $P \leq .1$; * $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$; NS $P > .1$; NA: non-significant ($P > .1$) and

interaction term removed from model

Figure Legend

Online Resource, Fig. 3 Nitrogen (N) dynamics during decomposition in upland versus wetland plots. Each symbol represents replicate litter bags containing leaf material of a single species from a single harvest and habitat collected during the first two years of decomposition (5 harvests total). Mean values of proportion of initial N remaining and proportion of initial mass remaining are displayed; lines are +/- standard error of the mean proportion of initial N remaining. The area above the dashed line indicates net N immobilization: litter N content at harvest exceeds initial litter N content. The area below the dashed line indicates net N mineralization: litter N content at harvest is less than initial litter N content. Symbol key: black = wetland plots; grey = upland plots.



Online Resource, Fig. 3