

Plant sex influences aquatic–terrestrial interactions

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Abstract. A growing body of research shows that plant genetic factors can influence ecosystem processes and structure communities, but one aspect that has received little study is sex differentiation in dioecious plants. Since headwater streams are reliant on riparian leaf litterfall, plant sex differences in leaf traits may influence in-stream processes. Sitka willow (*Salix sitchensis*) at Mount St. Helens is dioecious and heavily infested with the stem-boring weevil (*Cryptorhynchus lapathi*), which causes branch dieback and summer litterfall. We found that female willow shrubs tend to grow closer to the stream bank, are more likely to be infected by the weevil, and have 42% higher litter C:N than male willows. These factors may lead to increased litter inputs and slower litter mass loss for female willows. The combination of colonization location, herbivore attack, altered litter quality, and slower mass loss results in female shrubs providing more sustained carbon and nutrient resources to microbes and invertebrates in the early successional streams at Mount St. Helens. In addition, since dioecy is a relatively common trait in riparian habitats, it is possible that plant sex plays a far more interesting role in structuring linked terrestrial–aquatic communities and ecosystem processes than previously understood.

Key words: dioecious; disturbance; herbivory; leaf litter; litter mass loss; Mount St. Helens; plant sex; stream ecology; succession; volcano; weevil; willow.

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INTRODUCTION

Headwater stream communities and their productivity are highly dependent on inputs of organic matter and nutrients from riparian tree leaf litterfall (Wallace et al. 1997). The quantity and quality of leaf litter vary by tree species, but can also vary greatly within a species to influence in-stream decomposition and ecosystem processes (LeRoy et al. 2006, 2007, Jackrel et al. 2016). Leaf litter of high quality tends to be relatively rich in nitrogen and/or phosphorus, and supports productive communities of detritivores.

Rapid breakdown of labile leaf litters results in higher turnover of nutrients and organic matter and accelerated transfer of these materials to higher trophic levels, but recalcitrant litters can provide detrital resources for longer periods of time (Kaushik and Hynes 1971). The timing of leaf litterfall during the year may also affect the availability of nutrients and organic matter to detritivores in conjunction with hydrologic conditions that may or may not allow for the retention of leaf litter in the stream.

Terrestrial herbivores, including insect defoliators, often influence the chemistry and timing of

leaf litter inputs to terrestrial systems (Risley and Crossley 1993) and may influence adjacent aquatic ecosystems reliant on organic matter inputs (Cebrian and Lartigue 2004). Plants attacked by insect herbivores may increase lignin and defensive compounds (tannins, terpenes, alkaloids), which can reduce the overall quality of leaves. Most deciduous plants in temperate forests drop their leaves in the late autumn months, but some herbivores can induce leaf drop throughout the growing season as well, altering the seasonality of detrital inputs to ecosystems. Herbivores can attack various plant structures (leaves, stems, branches) and thus alter the deposition of both leaves and woody debris.

Sitka willow shrubs (*Salix sitchensis* Sanson ex Bong.) have been one of the major colonizers of riparian habitat on the Pumice Plain at Mount St. Helens (Lawetlat'la in Cowlitz; Washington, USA; del Moral and Jones 2002). Willows are heavily infested with the invasive stem-boring weevil (*Cryptorhynchus lapathi* L.; Coleoptera: Curculionidae; Broberg et al. 2002). The weevil attacks the base of willow stems, which often causes leaf death and sometimes leads to whole branch dieback or whole-plant mortality (Che-Castaldo et al. 2019).

Willow species are often dioecious, where individual shrubs can be either male or female. Many dioecious plants show skewed sex ratios across the landscape (Bierzychudek and Eckhart 1988, Elmquist et al. 1988a, Sinclair et al. 2012), with many populations skewed male (Hultine et al. 2016). Riparian habitats can contain high proportions of dioecious species (32%; Freeman et al. 1980), and previous studies show either no difference for willow male:female sex ratios or in some cases ratios that are skewed female due to higher resource availability and the needs of female plants to produce costly reproductive structures (Hultine et al. 2007, 2018). Riparian systems tend to have higher nutrients and moisture than upland systems, which could also alter litter quality and potentially influence herbivore susceptibility or preferences (Danell et al. 1985, Elmquist et al. 1988b, Boecklen et al. 1990, Cornelissen and Stiling 2005). Male and female plants can differ in terms of water use, nutrient use, plant chemistry, herbivory, and mycorrhizal colonization (Varga et al. 2013, Vega-Frutis et al. 2015). At Mount St. Helens, sex ratios are skewed

female (2:1) across both upland and riparian habitats (Che-Castaldo et al. 2015). Although many studies have explored how plant sex influences plant phenotypes, few studies have explored how these factors may influence extended phenotypes (Whitham et al. 2006) and ecosystem functions like nutrient cycling and decomposition (Hultine et al. 2016). The effects of dioecy on traits that may alter ecosystem function have yet to become a major focus of gene-to-ecosystems research (Barrett et al. 2010). There are opportunities to study potential influences of plant sex and herbivory in both terrestrial and aquatic systems and in the context of predicted climate changes which may favor one sex over the other (Hultine et al. 2016).

In this study, we report on the interacting influences of willow dioecy and stem-boring weevil attack on willow growth location, litter quality, litter mass loss, and the potential influence these interactions may have on primary successional stream communities and ecosystem processes at Mount St. Helens. We hypothesized that female willow plants would (1) colonize closer to stream edges, providing a stronger influence on stream ecosystems, (2) have higher attack rates by the stem-boring weevil, which may increase summer mortality and litterfall, (3) have lower quality leaf litter, specifically lower nutrients and higher defense compounds, (4) demonstrate slower in-stream leaf litter mass loss, and (5) result in increased colonization by litter-dwelling aquatic macroinvertebrates based on the longer-lasting detrital resource.

MATERIALS AND METHODS

Site description

The 1980 eruption of Mount St Helens transformed over 600 km² of forested area from a combination of pyroclastic flows, lahars, hot air blast, and tephra fall. The most disturbed area, besides the crater itself, was the pyroclastic flow zone and an area commonly called the Pumice Plain. This 15-km² area was first buried by over 100 m of pumice, ash, and sand in one of the largest terrestrial landslides in recorded history (2.8-km³ debris avalanche; Lipman and Mullineaux 1981), then hit with a hot lateral blast of flying rock debris, and finally covered in 0.3 km³ of lava which was as much as 40 m thick in

places (Swanson and Major 2005). Forests and stream systems flowing north through secondary temperate rainforest into Spirit Lake and the Toutle River drainage were obliterated. In the years following the eruption, springs and seeps re-established and runoff from snowmelt created a variety of new perennial and seasonal channels through the Pumice Plain (Fig. 1). Since 1980, vegetation has slowly colonized the Pumice Plain and several small streams are developing significant riparian tree cover. The species that dominate riparian habitats are Sitka willow and green alder (*Alnus viridis* (Chaix) DC.; del Moral and Jones 2002). Across the Pumice Plain, willow shrubs have been regularly attacked by a stem-boring weevil (*C. lapathi*) since at least 1989,

when they were first recorded (Che-Castaldo et al. 2019).

Field data collection

In May 2018, we selected individual willows within 10 m of the stream edge (in belt transects) along two separate streams, Goose Creek and Geo-West Creek, on the Pumice Plain (Fig. 1). We haphazardly selected discrete individuals for which we could determine sex. Individual shrubs selected tended to be large and had stems that were not mixed with neighboring plants. We selected 104 individual willow shrubs and tagged one or more robust, healthy branches on each individual as either male or female based on the presence of reproductive structures. In

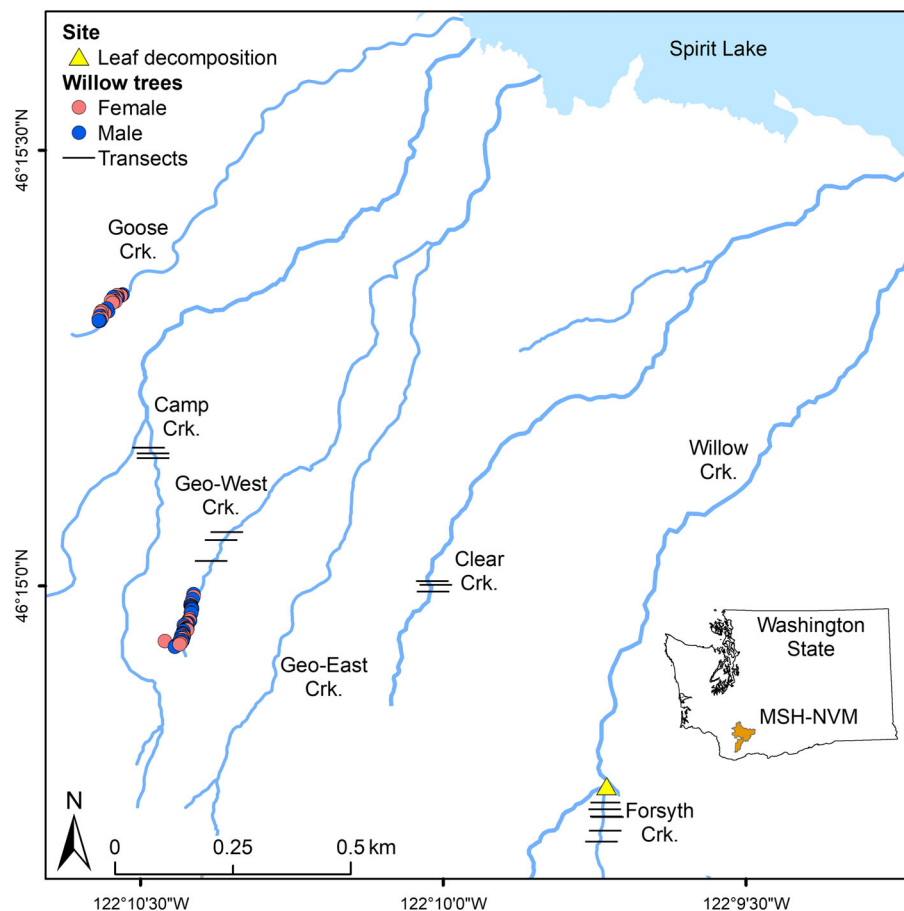


Fig. 1. Map of the Pumice Plain study area in the Mount St. Helens National Volcanic Monument, WA, showing locations of female (pink circles) and male (blue circles) willow shrubs (*Salix sitchensis*) in belt transects along Goose Creek and Geothermal West Creek. Line-point intercept transect locations are shown as black bars. The leaf litter decomposition experiment occurred in Forsyth Creek (yellow triangle).

July 2018, we returned to the two sites and measured the straight-line distance between each tagged shrub and the streamside (wetted edge). For each shrub, we made note of weevil infection, branch dieback, and shrub mortality. We collected dry leaf litter from dead branches on weevil-attacked male and female shrubs for litter chemistry and decomposition analyses. Additionally, to determine willow cover and to gather an additional data set on distance to the stream edge, we created line-point intercept transects at 16 locations across the Pumice Plain (Fig. 1). Each transect ran perpendicular from the stream edge to the furthest willow within 15 m. Along the entire transect, we noted the overhead vegetation and measured the overlapping cover for every willow stem. For each stem, we used its cover midpoint to measure its distance from the stream edge and we recorded willow sex.

Leaf litter chemistry

Leaf litter from male and female willow shrubs was collected in separate paper sacks for each individual and air-dried in the laboratory. Subsamples (0.50 g) were freeze-dried (Millrock Technology, Kingston, New York, USA), ground to a homogeneous consistency (KRUPS Type F203), and used to measure a variety of litter chemical traits. Subsamples (25 mg) of freeze-dried materials were extracted for soluble condensed tannins with 70% acetone and 10 mmol/L ascorbic acid. We used the butanol-HCl method to determine soluble condensed tannin concentrations (Porter et al. 1986), with standards purified from Sitka willow following the methods of Hagerman and Butler (1989). We measured absorbance at 550 nm on a spectrophotometer (SpectraMax 384; Molecular Devices, San Jose, California, USA). Additional subsamples (2 mg) were weighed into tin capsules (5 × 8 mm) to determine %C, %N, and C:N on an elemental analyzer (2400 CHNS/O Series II System, Perkin Elmer, Waltham, Massachusetts, USA).

Leaf litter mass loss

Senescent summer leaf litter from male and female willow shrubs attacked by weevils was collected from Goose Creek on 17 July 2018. Litter from each shrub was kept separate, air-dried, weighed into 2-g quantities, and placed into

mesh bags (4-mm mesh openings). We determined handling losses by transporting each litterbag in a separate paper sack, weighing any leaf particles that remained, and subtracting handling losses from initial weights. We placed litterbags into several small pools in the Forsyth tributary of Willow Creek. We chose Forsyth due to its steady flow conditions throughout the summer and its rich litter-dwelling community. We attached litterbags to rope in the stream using cable ties and incubated them for 0, 9, and 43 d ($n = 3/\text{sex}/\text{date}$). We collected litterbags on each harvest date by placing them into polyethylene zipper bags, returning them to the laboratory on ice, and processing them within 24 h. We rinsed leaves with deionized water and then froze remaining leaf mass at -80°C followed by lyophilization for 72 h. We collected benthic macroinvertebrates (BMI) from each litterbag by sieving the rinse water through a 250- μm net and preserving all material in 70% ethanol. We sorted invertebrates under dissecting microscopy (40 $\times$) and identified each individual to the lowest taxonomic level possible (Merritt et al. 2008). We weighed the lyophilized leaf material from each litterbag for dry mass to the nearest 0.0001 g, then combusted a 25 mg subsample at 550°C for 1 h, and weighed the subsample again to determine ash-free dry mass (AFDM, g). We determined decomposition rates (k) using the $\ln$ of AFDM (g) regressed against day in the stream (Jenny et al. 1949).

Statistical analysis

We assessed variables for the assumptions of normality and equality of variances and used $\ln$ -transformation when necessary. To compare willow distances from stream edge, we used belt transect data and a two-way ANOVA with explanatory variables of willow sex (male or female), stream (Geo-West or Goose), and their interaction ($n = 104$ plants). We also used data from line-point intercept transects and t -tests to determine whether willow sex influenced the distance between individuals and the stream edge or total willow cover (16 transects, $n = 124$ stems). To examine the frequency of willows infected by weevils, we used logistic regression with explanatory variables of willow sex (male or female), stream (Geo-West or Goose), and their interaction ($n = 104$ plants). Of those shrubs

with weevils (61 willows; 34 from Geo-West Creek and 27 from Goose Creek), we used the same logistic regression to compare the frequency of branch mortality. We used t -tests to compare leaf litter chemistry between the sexes. To examine leaf litter mass loss, we used a two-way analysis of covariance (ANCOVA) to compare the ln-transformed AFDM remaining (g) through time (harvest days 0, 9, 43), and between male and female willows, and their interaction ($n = 18$ samples). To examine relationships between macroinvertebrate species diversity index values and litter quality parameters, we used simple linear regression. All parametric analyses were run in JMP (Pro 14; SAS Institute, Cary, North Carolina, USA). For macroinvertebrates, we calculated Simpson's diversity index (D') per litterbag and made comparisons among communities using multi-response permutation procedures (MRPP) grouped by willow sex and non-metric multidimensional scaling ordination (Bray-Curtis distance measure) in PC-Ord (6.0, McCune and Mefford, MjM Software, Gleneden Beach, Oregon, USA).

RESULTS

We found similar results using both belt transects and line-point intercept transects comparing the distributions of female and male willows along streams of the Pumice Plain of Mount St. Helens. When comparing male and female willow shrubs in 10-m belt transects along two headwater streams on the Pumice Plain, we found that female willows tend to grow 27.9% closer to the stream bank than males ($P = 0.0244$). The average distance to the wetted edge for female willows was 4.04 m (95% confidence interval (CI): 3.46–4.63), while the average distance for males was 5.15 m (95% CI: 4.56–5.79). Out of 104 willows sampled, the two sexes were nearly equally measured along both streams (50% female along Geo-West Creek, 55% female along Goose Creek), and there was no significant effect of stream ($P = 0.3478$) or the sex $\times$ stream interaction on the distance to the wetted edge ($P = 0.4976$). Using line-point intercept transects accounting for every stem from stream edge to the edge of the riparian zone, we again show that female willow shrubs grow significantly closer to the stream edge than male

willows ($t_{(122)} = 2.56$, $P = 0.0118$). The average distance to the wetted edge for female willows was 76% closer than for males (2.50 m; 95% CI: 1.73–3.27), while the average distance for males was 4.38 m (95% CI: 3.17–5.58). Summing all willow cover across each transect, there was 53% higher female willow cover (2.43 m²) than male (1.14 m²) across all streams ($t_{(27)} = -2.12$, $P = 0.0431$).

In the belt transects, female willow shrubs were more likely to be infected by the stem-boring weevil (*C. lapathi*) than males ($\chi^2 = 6.82$, $P = 0.0090$), but there was no difference in infection rates between the two creeks ($\chi^2 = 2.29$, $P = 0.1303$). There was a significant interaction between sex $\times$ stream ($\chi^2 = 4.11$, $P = 0.0426$) which showed that infection differed more strongly by sex at Goose Creek than at Geo-West Creek. The majority of female willow shrubs were infected with weevils at Goose Creek (86%), but only 56% of females were infected at Geo-West Creek, compared with 44% and 50% of male willow shrubs along both creeks, respectively. Weevil-infected willows may or may not show branch or complete mortality, especially because willows near water may be better able to survive weevil attacks. Comparing male and female willows that were infected by weevils, we found that early season branch mortality did not differ by sex, stream, or their interaction ($P > 0.5142$). Of the 61 willow shrubs infected by weevils, 37 were female (14% with branch die-back) and 24 were male (20% with branch die-back).

Leaf litter chemistry differed between the willow sexes. Male leaf litter was significantly higher in % nitrogen ($t_{(7)} = 2.74$, $P = 0.0290$; Fig. 2A) and showed a significantly lower C:N ratio ($t_{(7)} = -2.49$, $P = 0.0411$) than female litter (Fig. 2C). The two litter types did not differ in % carbon ($t_{(7)} = -0.46$, $P = 0.6621$; Fig. 2B) or condensed tannins ($t_{(7)} = 0.95$, $P = 0.3714$; Fig. 2D).

Female willow litter lost less mass than male litter over the course of 43 d in the stream (Fig. 3A). There was a significant effect of sex ($P = 0.0100$) and days in the stream ($P < 0.0001$) on ln-AFDM, but a non-significant interaction term ($P = 0.2341$) which means the effect of plant sex on overall decomposition rates was weak. Exponential mean decay rates were -0.0150 (0.0017 SE) for males and -0.0131 (0.0004 SE) for

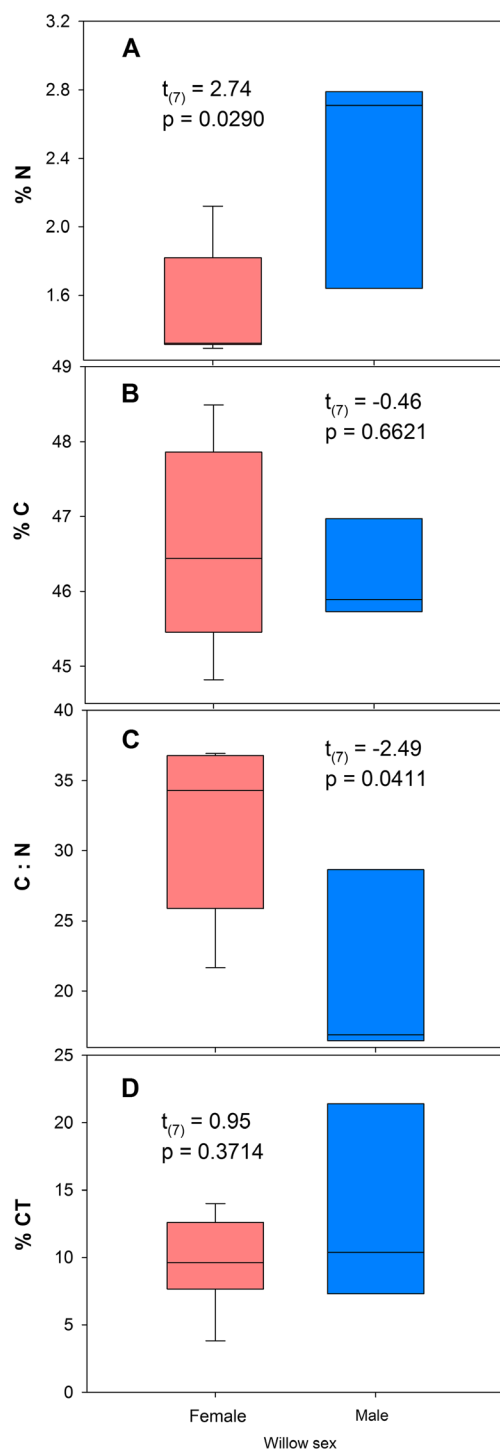


Fig. 2. Leaf litter chemistry differences between female (pink) and male (blue) willows (*Salix sitchensis*). Box plots show median values for (A) % nitrogen (N), (B) % carbon (C), (C) C:N ratio, and (D) condensed

female. After 9 d in the stream, female willow leaves had lost an average of 24.8% AFDM, while male leaves had lost an average of 33.6%. After 43 d, female leaves had lost an average of 68.3% AFDM and male leaves had lost an average of 77.1%.

During the decomposition process, the chemical content of the litter changed, but differentially for male litter and female litter. Both sexes accumulated nitrogen in leaf tissues, likely through microbial colonization, but male litter, which was higher in %N to begin with, stayed higher in %N throughout the study (Fig. 3B; sex $P = 0.0017$, days $P = 0.0002$, sex $\times$ days $P = 0.4183$). Female litter, which had a higher C:N content initially, showed a more rapid drop in C:N over time (Fig. 3C; sex $P = 0.0010$, days $P = 0.0004$, sex $\times$ days $P = 0.1240$). Since the %C content of both litters stayed relatively constant ($P = 0.3906$), it is possible that female litter accumulates N more rapidly than male litter, and over time, the C:N ratios of the leaf litter may converge.

Benthic macroinvertebrates that colonized litterbags did not discriminate much between male and female leaves. There was no significant difference in BMI communities between male and female leaves (MRPP $A = -0.0099$, $P = 0.6114$), nor significant effects on BMI abundance ($t_{(10)} = 0.4466$, $P = 0.6646$). There were, however, influences of leaf litter chemistry on BMI species diversity. Simpson's diversity index (D') increased significantly as increasingly more AFDM was lost ($F_{(1,10)} = 11.59$, $P = 0.0067$), which may have simply reflected increased species accumulation through time. However, there was also a significant decrease in Simpson's D' with increased litter C:N ($F_{(1,10)} = 5.18$, $P = 0.0461$).

DISCUSSION

The introduced stem-boring weevil is altering the inputs of leaf litterfall to streams at Mount St. Helens, specifically by increasing summer

(Fig. 2. Continued)

tannins (CT). Box outlines show 25% and 75% quantiles, and error bars represent minimum and maximum values. Student's t -test and P -values presented on the figures.

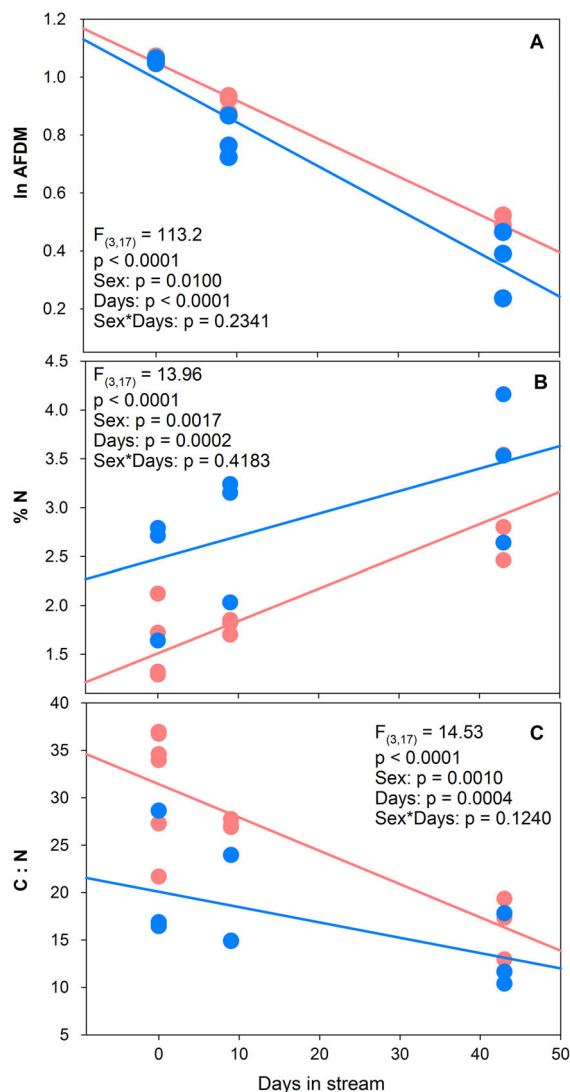


Fig. 3. Changes in leaf litter ash-free dry mass (AFDM) and litter chemistry through time for female (pink) and male (blue) willows (*Salix sitchensis*). (A) Leaf litter mass loss (ln-AFDM) is influenced by plant sex (male vs. female), and days in the stream, but not their interaction. (B) Percent nitrogen (% N) of willow litter is influenced by plant sex, days in the stream, but not their interaction. (C) C:N content of willow litter is influenced by plant sex, days in the stream, but not their interaction. Results of ANCOVA are presented on the figures.

litterfall, causing branch mortality, and by preferentially attacking females, which tend to grow closer to streams (Bierzychudek and Eckhardt 1988). These results are similar to some studies

(Lovett Doust and Lovett Doust 1985), but differ from others, which have shown male willow leaves were more frequently attacked by insect herbivores (Alliende 1989). These factors in combination are causing a shift in the seasonality of organic matter dynamics on the Pumice Plain, which has consequences for carbon cycling and the activity of in-stream litter-dependent communities in this early successional system. Willows in this system are producing biomass that is rapidly cycled back into the soil organic matter and into the atmosphere as CO₂ through both terrestrial decomposition and aquatic decomposition of summer-fall leaves and dead branches. Since females are growing closer to streams, have higher overall canopy cover in riparian forests, and have summer-input litter with a higher C:N content, female willows are contributing more leaf litter to streams and that litter is more recalcitrant and functions as a resource longer in the stream.

Female leaf litter is lower quality (lower % N, higher C:N) as has been shown by several other studies (Alliende 1989, Boecklen et al. 1990, Elmquist et al. 1991), which would likely lead to larger differences in litter mass loss had this study been larger in scope (increased sample sizes). Despite being of lower quality, female litter accumulates nitrogen faster than male litter once incubated in the stream, possibly due to differential colonization by aquatic fungi and bacteria. Other causes for the discrepancies in litter quality could be environmental, since females are growing closer to water sources in this extreme environment. In addition, litter quality differences could be due to differential resource allocation and female requirements to make flowers, fruits, and seeds that have been shown to decrease allocation to defensive compounds like tannins and salicortin (Elmqvist et al. 1991). We found no statistically significant differences in condensed tannins between males and females, and it is thought that weevil attack is not strongly influenced by willow defensive compounds (Che-Castaldo et al. 2019). In this system, weevils may be keying into differences in willow cambium nutrient content (Che-Castaldo et al. 2019) or olfactory cues based on other chemical compounds as has been shown for this weevil on poplars (Broberg and Borden 2005).

Interestingly, there are spatial patterns in weevil infection that may also be influencing this aquatic-terrestrial interaction. Our results corroborate those of Che-Castaldo et al. (2019) which showed that female riparian willows were more frequently attacked by the weevil. We also found a significant sex $\times$ stream interaction with a higher proportion of female willows attacked by weevils at Goose Creek than at Geo-West Creek. We have surveyed all five streams across the Pumice Plain and are exploring other environmental variables that may drive riparian plant colonization and in-stream processes (C. J. LeRoy et al., *unpublished manuscript*), several of which may also influence willow growth, flowering, and weevil attack, but we have not yet narrowed in on a causative factor. Possible factors include host plant vigor or stress (Che-Castaldo et al. 2019), and the environmental factors that influence vigor and stress, such as water or nutrient availability and the ephemerality of surface water. Based on several years of observations, Goose Creek is more ephemeral than Geo-West Creek and is often dry late in the summer (C. J. LeRoy et al., *unpublished data*) and especially senescent-feeding larval cohort weevils at this site may be responding to seasonal willow water stress (Che-Castaldo et al. 2019).

These findings of plant sex and herbivory effects on willow shrub distributions and organic matter dynamics may influence how new streams are developing on the Pumice Plain post-eruption. The 1980 eruption of Mount St. Helens resulted in the creation of four new stream drainages. The colonization of willows along channels influences channel stability, stream temperature, and inputs of organic material (Kaushik and Hynes 1971, Wallace et al. 1997). In addition, since these riparian willow zones are dominated by female plants, they may be receiving relatively more summer-input organic matter. Some outstanding questions that remain in this system are as follows: (1) Are male and female litters colonized differentially by in-stream microbes? (2) How does the shift in litter seasonality alter successional processes? (3) How does the domination of streams by female willows change successional trajectories? (4) How does plant sex otherwise structure early successional landscapes?

The study of plant sex extends the field of genes-to-ecosystems research beyond the

influence of general genetic variation within individuals (Schweitzer et al. 2004, Whitham et al. 2006) to the influence of male and female individuals within species (Hultine et al. 2016). The influences of dioecy on ecosystem function need further exploration, especially since the expression of sex in dioecious plants is not straightforward (Rowland et al. 2002, Zhou et al. 2018). Additionally, understanding the influence of sex on ecosystem processes is crucial as climate and landscapes change since plant sexes may be differentially challenged by shifting climatic regimes (Hultine et al. 2016).

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DATA AVAILABILITY

Data are available at GitHub: https://github.com/carrileroy/MSH_2018.