Title

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Keywords

Abstract

Introduction

Research Questions

1. What is the relative importance of genotype vs. the environment in determining plant phenotype?
2. What is the relative importance of genotype vs. the environment in determining associated above- and belowground communities?
3. Which plant phenotypes are associated with above- and belowground community responses?

Methods

*Study Site*

We conducted this research at Lanphere Dunes (40**°** 53'29.85"N, 124**°** 8'49.06"W), a pristine coastal dune ecosystem managed by US Fish and Wildlife service in Humboldt County, California. Coastal willow (*Salix hookeriana* ex Barratt ex Hooker) naturally occurs in nearshore dune swales – seasonal freshwater wetlands that form in depressions between dune ridges (Pickart 2009). Aside from coastal willow (hereafter willow), the dominant vegetation in these swales consists of beach pine (*Pinus contorta* ssp. *contorta*) and slough sedge (*Carex obnupta*).

*Experimental Design*

Prior to bud burst in February 2012, we took shoot cuttings (40 cm length & ~0.5 cm diameter) from one to two replicates of 10 different willow genotypes from a pool of 26 locally collected willow genotypes planted in a large common garden experiment. Details about the establishment of this common garden are given in Barbour et al. (2015). These 10 genotypes displayed substantial variation in both leaf quality and plant architectural traits (Barbour et al. 2015). Shoot cuttings were soaked in water overnight and then planted in a mixture of 80% perlite, 20% peat moss and \_\_\_ dolomite lime inside 'cone-tainers'. We grew cuttings under ambient weather conditions outside the greenhouse at Humboldt State University.

Wind experiment – In late May 2012, we planted 200 willow cuttings in a split-plot experimental design at Lanphere Dunes. At 10 different willow patches (blocks), we established an 'exposed' and 'unexposed' common garden. Each garden consisted of one replicate cutting of each of 10 genotypes randomly planted in 2 m by 0.5 m grid with 0.5 m spacing between plants. The center of exposed and unexposed gardens within each block were the same distance (2 m) from the edge of the willow patch to control for insect accessibility; however, exposed gardens faced prevailing winds during the growing season. To estimate the maximum amount of wind speed (km/h) experienced by exposed vs. unexposed plants, we went out on a windy afternoon in September 2012 (weather station estimated wind speeds of 22 km/h during this period) and used an anemometer (MAKE and MODEL) to measure wind speed at a height of 37 cm aboveground (approximate height of tallest plants in the garden) in each plot of our experiment. Maximum wind speed measurements were taken over a 30 s period and haphazardly started on either an exposed or unexposed site. On this windy day, exposed treatments experienced 5-fold higher wind speeds on average compared to unexposed treatments (paired t-test: *t9* = 13.7, *P* < 0.001).

Ant-aphid experiment – We established common gardens around 5 different ant mounds (blocks) in late May of 2012. Within each block, we randomly planted 20 cuttings (aphid and control treatment of each of 10 genotypes) with 0.5 m spacing at each distance of 1, 6, and 12 meters from the edge of the ant mound, for a total of 60 cuttings per ant mound (300 cuttings for entire experiment). We removed all arthropods on the willows at the time of planting. On May 22, we collected aphids (*Aphis farinosa*) and placed 5 adult apterate aphids on willow cuttings in the aphid treatment. We bagged aphids onto the apical shoots of cuttings using organza bags to promote aphid establishment in spite of oncoming inclement weather (wind and rain). We also placed organza bags on all control plants as well. On May 27, we checked aphid treatments to ensure there were 5 adult aphids and removed bags from all cuttings. If necessary, we added aphids to these treatments until there were 5 adults and we removed any aphid nymphs that were produced since initial establishment. We checked plants for aphids on June 6, June 13, June 24, July 4, July 14, and July 20, 2012. On May 27, we double-checked willows to ensure that all arthropods (spiders and leaf rollers; except for a couple of stem galls) were removed. Up until May 27, we supplemented planted cuttings with water to promote the survival of cuttings. The ant-aphid experiment was restricted to the summer of 2012, because in the summer of 2013 there was high willow mortality (\_% of plants died by DATE) and *Aphis farinosa* was not in high enough abundance on naturally occurring willows to repeat the aphid treatment.

*Measuring willow phenotypes*

Plant architecture – To measure plant architecture, we measured plant height, the number of shoots produced, and total shoot growth at the end of each growing season (\_\_ 2011 and \_\_ 2012). We quantified plant height as the distance (mm) from the ground to the tip of the tallest shoot. We quantified total shoot growth by measuring every shoot on each plant to the nearest millimeter and summing the total shoot growth for each plant.

Leaf quality – We measured leaf quality in several ways.

We measured plant quality in several ways. First, we have correlational data on the concentration of 29 phenolic glycosides, total condensed tannins and carbon:nitrogen ratios and the abundance of one of a dominant herbivore Caloptilia pruniella that specializes on willows from the willow common garden in which we took cuttings from. We knew that \*Caloptilia pruniella\* abundance varied across these genotypes [NEED TO CONTROL FOR PLANT SIZE AND CONVERT TO DENSITY] so we looked for correlations between these plant traits and the abundance of this herbivore. Furthermore, we examined whether the rank average abundance of *C. pruniella* on these 10 genotypes in the common garden matched our observed variation in *C. pruniella* abundance on genotypes in unexposed plots.

Second, we conducted leaf quality assays using Spodoptera exigua. We replicated this experiment twice, once with late first instars (4 day duration) and another with 2nd instar larva (3 day duration). For both experiments, we added a single freshly collected leaf to a 30 mL plastic transport vials (loosely capped at end) with moist cotton ball, and then we added one, randomly selected larva to each vial using a small paintbrush. We used wet body mass at the end of each experiment as our measure of larva performance and therefore leaf quality (i.e. small larva = low quality plant tissue

We measure several putatively important traits that could shape leaf quality for herbivores, including water content, trichome density, percentage carbon (C) and nitrogen (N), and C : N. For water content and trichome density, we excised a single fully expanded and undamaged leaf from each plant in MONTH YEAR. We placed leaf samples into separate plastic bags with a moist paper towel, within a cooler and immediately brought them back to the laboratory. We then weighed leaves to obtain fresh mass (g), and oven-dried them at 60 °C for 72 h to obtain dry weight (g) (Cornelissen et al. 2003). We calculated leaf water content as the (Munns & PrometheusWiki Contributors 2010). To measure trichome density, we counted the number of trichomes along an 11 mm by 1 mm transect in the centre of the leaf, halfway between the leaf edge and the mid-vein, under a dissecting scope. To measure percentage C and N, we collected up to 3 fully expanded and undamaged leaves from each plant.

Leaves were air-dried and grounded to a fine powder using a ball mill (Mixer/Mill 8000D, SPEX SamplePrep; Metuchen, NJ, USA). Subsamples of each material were then analysed for percentage C and N on an elemental analyser (ECS 4010; Costech Analytical Technologies, Valencia, California, USA) using atropine (4.84% N and 70.56% C) as a reference standard.

*Quantifying Aboveground Community*

Given the small size of our plants (max plant height = \_\_ cm) and to avoid sampling arthropods on surrounding vegetation, we visually surveyed plants for arthropods to determine the abundances of different (morpho)species. Given the relatively low abundances of individual morphospecies, we grouped arthropods at the Family-level for insects and at the Order-level for other arthropods. JUSTIFY GROUPINGS. For the wind experiment, we surveyed arthropods once at the end of July 2012 and then once a month in May, June, and July of 2013. For the ant-aphid experiment, we surveyed arthropods on 5 different occasions between early June and late July 2012. ALSO MENTION HOW I’M TAKING THE MAXIMUM OBSERVED ABUNDANCE OF EACH ARTHROPOD SAMPLED OVER THE COURSE OF EACH YEAR.

*Quantifying Belowground Community*

At the end of the growing season in 2013, we dug up the willows from the wind experiment in order to sample the mychorrizal and bacterial communities associated with the willow roots. We did not sample the belowground communities of plants in the ant-aphid experiment due to the high mortality of plants in 2013. To sample these belowground communities, we carefully removed dirt until we found all of the living root tissue. We then placed the root tissue of each plant into separate plastic bags that went into a cooler, which were immediately transported back to the lab and kept at TEMPERATURE until further processing.

ASK SONYA FOR DETAILS ON QUANTIFYING THE BELOWGROUND COMMUNTIES.

*Soil characteristics*

Soil nutrients, total organic matter, and moisture may all influence the assembly of mychorizzal and bacterial communities on plant roots (cite). Moreover, we expected that wind exposure to affect these soil characteristics (cite); therefore, we measured soil nutrients, total organic matter, and moisture within each replicate common garden.

To estimate soil nutrient uptake by willows, we installed Plant Root Simulator (PRS) Probes (Western Ag Innovations, Saskatchewan, Canada) at three randomly selected locations within each garden for 11 days in September 2012. PRS Probes estimate nutrient supply rates to roots by continuously adsorbing charged ionic elements over the burial period. For our study, we estimated potential root uptake of total N, NO3+, NH4-, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, and Cd.

To measure total organic matter content (TOM), we used a trowel to collect soil (depth = 0 – 15 cm) adjacent to the randomly positioned PRS probes in September 2012. Soils were transported back to the lab in plastic bags, sieved into fragments less than 2 mm, randomly subsampled using a soil splitter, and dried at 105 **°**C for 72 hours. We then weighed a subsample of the oven dried soil into an oven dried crucible and placed the crucible and soil into a furnace to be combusted at 375 **°**C for 16 hours. We then weighed the combusted samples, placed them in a desiccator for 20 minutes, and weighed them again. We calculated percent organic matter as .

To measure soil moisture (volumetric water content, m3/m3), we used a 5TE soil sensor coupled to an EM50 Digital/Analog Data Logger (Decagon Devices, Pullman, Washington, USA). In September 2012, while PRS probes were in the ground, we measured soil moisture at a depth of 5 cm in three random locations within each garden on three different days between 1100 – 1500 hours. We repeated this same sampling scheme in early July 2013.

*Statistical Analyses*

For both experiments, we used mixed-effect models to analyze responses measured at the plant level (e.g. willow phenotypes, above- and below-ground communities). For the wind experiment, wind exposure, willow genotype, and their interaction were set as fixed effects, whereas block and wind exposure nested within block were set as random effects. For the ant-aphid experiment, aphid presence, distance to ant mound, willow genotype, and all possible interactions were treated as fixed effects, whereas block and distance to ant mound nested within block were treated as random effects.

Willow phenotypes – To examine how wind exposure and willow genotype affected plant architecture and leaf quality, we used permutational ANOVA (PERMANOVA) (Anderson 2001). We standardized traits (mean = 0, SD = 1) to give them the same weight in our analyses. We then conducted separate PERMANOVAs on the pairwise Euclidean distances of traits associated with plant architecture (X traits) and leaf quality (Y traits).

Above- and below-ground communities – As with the willow phenotypes, we used PERMANOVA. Specifically, we conducted separate PERMANOVAs on the pairwise Bray-Curtis dissimilarities of the composition of arthropod, myrchorrizal, and bacterial communities.

To examine the effect of wind exposure on soil nutrients, we first standardized nutrients (mean = 0, SD = 1) to give them equal weight in the analysis. We then performed a PERMANOVA on pairwise Euclidean distances, where wind exposure was a fixed effect and ‘block’ was a random effect. To test for an effect of wind exposure on soil organic matter and moisture, we used separate paired t-tests (paired by block).

All analyses were conducted in R version \_\_ (R Core Team YEAR).

Results

Wind experiment:

*Willow Phenotypes:* There was substantial variation in willow growth among willow genotypes and between exposed and unexposed trees.

Discussion

Acknowledgements

References

Figure Legends

**Tables**

**Table 1**: Wind experiment – Permutational multivariate ANOVAs on willow phenotypes (plant architecture and leaf quality) and willow community responses (arthropods, mycorrhizal, bacteria).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 2012 | | | 2013 | | |
| Response | Treatment | *df* | *F* | *P* | *df* | *F* | *P* |
| Plant architecture | Genotype (G) |  |  |  |  |  |  |
|  | Wind exposure (E) |  |  |  |  |  |  |
|  | G x E |  |  |  |  |  |  |
| Leaf quality | Genotype (G) |  |  |  |  |  |  |
|  | Wind exposure (E) |  |  |  |  |  |  |
|  | G x E |  |  |  |  |  |  |
| Arthropod community | Genotype (G) |  |  |  |  |  |  |
|  | Wind exposure (E) |  |  |  |  |  |  |
|  | G x E |  |  |  |  |  |  |
| Mychorrizal community | Genotype (G) | -- | -- | -- |  |  |  |
|  | Wind exposure (E) | -- | -- | -- |  |  |  |
|  | G x E | -- | -- | -- |  |  |  |
| Bacterial community | Genotype (G) | -- | -- | -- |  |  |  |
|  | Wind exposure (E) | -- | -- | -- |  |  |  |
|  | G x E | -- | -- | -- |  |  |  |

**Table 2**: Ant-aphid experiment: Permutational multivariate ANOVAs on willow phenotypes (plant architecture and leaf quality) and willow community responses (arthropods, mycorrhizal, bacteria).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | 2012 | | |
| Response | Treatment | *df* | *F* | *P* |
| Plant architecture | Genotype (G) |  |  |  |
|  | Aphids (Eaphid) |  |  |  |
|  | Ant mound distance (Eant) |  |  |  |
|  | G x Eaphid |  |  |  |
|  | G x Eant |  |  |  |
|  | Eaphid x Eant |  |  |  |
|  | G x Eaphid x Eant |  |  |  |
| Leaf quality | Genotype (G) |  |  |  |
|  | Aphids (Eaphid) |  |  |  |
|  | Ant mound distance (Eant) |  |  |  |
|  | G x Eaphid |  |  |  |
|  | G x Eant |  |  |  |
|  | Eaphid x Eant |  |  |  |
|  | G x Eaphid x Eant |  |  |  |
| Arthropod community | Genotype (G) |  |  |  |
|  | Aphids (Eaphid) |  |  |  |
|  | Ant mound distance (Eant) |  |  |  |
|  | G x Eaphid |  |  |  |
|  | G x Eant |  |  |  |
|  | Eaphid x Eant |  |  |  |
|  | G x Eaphid x Eant |  |  |  |

Plant survival: end of 2012 and 2013

Plant architecture traits: height (2012 and 2013), total shoot growth (2012 and 2013), mature leaf count (2012 and 2013), number of shoots (2012 and 2013), mean shoot length (2012 and 2013). Need to think about whether I should set a lower limit on shoots for contributing to branching architecture…Look for correlations and a composite measure of plant architecture.

Plant quality traits: SLA (2012 and wind 2013), water content (2012 and wind 2013), trichome density (2012), spodoptera leaf quality (wind 2013), C:N (wind 2013)

Arthropod community responses: Total spider abundance, Total predator abundance, total ant abundance, total arthropod abundance, Caloptilia sp. (LTF, tent mines, etc.), tortricid moths (leaf-edge silk and rolls into leaf bundles), sawfly larva, multiple aphid species, psyllids, leaf hoppers, gallers, spiders, ants, other.

Herbivory: wind 2013 (includes details from different sources as well).

Fungal community responses: Ask Sonya (wind 2013).

Soil: organic matter content (wind 2012), nutrients (wind 2012), moisture/temperature/EC (wind 2012 and both 2013). Can only test for environmental treatment effects here. Look for correlations in wind 2012 dataset.

What to do with perfectly correlated random effects or zero variance parameters?

Experimental designs and modeling points.

Wind experiment is a split-plot design with wind exposure as the whole-plot factor and genotype as the split-plot factor. Note also that I attempted first to fit a random slope and intercept model (treatment | genotype), but that the correlation between the intercept and slope was +/- 1, suggesting that the model is more complex than the data can support. I am a bit surprised though, because I didn’t think I had too few levels of random effects. According to <http://glmm.wikidot.com/faq#singular_fits>, I should treat genotype as a fixed effect, but doesn’t that make the model more complex? Right now, it just seems to make more sense to treat it as a fixed effect and cross it with wind. Importantly though, since wind exposure is the whole-plot factor, I need to nest treatment within block (1 | block/treatment) in order to calculate the appropriate degrees of freedom for the effect of wind exposure.

Lastly, we measured several other plant traits that may influence plant tissue quality, including: specific leaf area, leaf dry matter content, trichome density, and percent leaf desiccation (i.e. browned portion at end of leaf tip). To measure these traits, we picked a single fully expanded leaf that appeared to be of the highest quality for that plant (method in summer of 2011).