Title

Abstract

Introduction

Research Questions

1. What is the relative importance of genotype vs. the environment in determining associated above- and belowground communities?
2. What are the mechanisms that are determining 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. The willow \*Salix hookeriana\* naturally occurs in nearshore dune swales - seasonal freshwater wetlands that form in depressions between dune ridges [@Pickart2009]. Aside from our study species, 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 \*Salix hookeriana\* genotypes from a pool of 26 unique genotypes planted in a large common garden experiment. Details about the establishment of this common garden are given in @CITATION. These 10 genotypes displayed substantial variation in a variety of plant traits FIGURES FOR SUPPLEMENTARY MATERIAL. 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.

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 \_ x \_ grid with \_\_ cm spacing between plants. The center of exposed and unexposed gardens within each block were the same distance (1.5 m?) from the edge of the willow patch to control for insect accessability; however, exposed gardens faced prevailing winds during the growing season. STATISTICS ON OVERALL WIND EXPOSURE OF PLANTS.

*Local Site Characteristics*

Wind exposure may alter local site characteristics such as soil nutrients as well as the rest of the plant community. Therefore, we characterized these aspects to identify the direct and indirect effects of wind exposure.

We used a 5TE soil sensor (Decagon Devices - Pullman, Washington, USA) to measure soil volumetric water content (m^3^/m^3^), temperature (C), and electrical conductivity (dS/m). We characterized the soil on 3 different days in early July, 2013 between 1130 and 1500 hrs.

*Soil Measurements*

At the end of the growing season (September 17 ‚Äì 28) in 2012, we measured X aspects of the soil, including: X nutrients, total organic matter content, moisture, temperature, and electrical conductivity.

To measure nutrient availability in the soil, we used Plant Root Simulator (PRS) Probes (Western Ag Innovations, Saskatchewan, Canada). We installed PRS probes at 3 randomly selected locations within each site for 11 days.

To measure total organic matter content (TOM), we used a trowel to collect soil at a depth up to 15 cm adjacent to the randomly positioned PRS probes on Sep 18, 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 an average temperature of 105 degrees Celsius for X days. 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 XX degrees Celsius for X days. We then weighed the combusted samples, placed them in a desiccator for 20 minutes, reweighed them. To calculate total organic matter we used the equation: TOM = (Oven Dry Weight -Combusted Weight)/Oven Dry Weight.

We used an EM50 Digital/Analog Data Logger (Decagon Devices, Washington, USA) to measure soil moisture, temperature, and electrical conductivity at a soil depth of about 5 cm. We measured soil moisture at 3 random locations within each site on three different days while PRS probes were in the ground. All readings occurred between GIVE HOURS.

*Soil*

In September 2012, we established soil probes to characterize nutrient availability at each site.

We also collected soil to characterize organic matter content of the different sites.

In addition, we measured soil moisture, temperature, and electrical conductivity in September 2012 and MONTH 2013.

*Plant community*

In MONTH 2013, we surveyed the percent cover of other members of the plant community growing in the sites.

*Plant Traits*

\*Plant Productivity\*: To quantify variation in plant productivity, we monitored total shoot growth over two growing seasons. 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 during each survey. We surveyed plants X times over the course of two growing seasons.

\*Plant Height\*: During plant productivity surveys, we also measured plant height. We quantified plant height as the distance from the ground to the end of the tallest shoot to the nearest millimeter. ALSO MEASURE ORIGINAL PLANT HEIGHT AND MEASURE THE DIFFERENCE IN PLANT HEIGHT OVER THE GROWING SEASONS.

\*Plant Quality\*: 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. NEED TO WORK ON METHODS. SEE SUPPLEMENTARY MATERIALS OF CRUTSINGER ET AL. 2006. FILL OUT PERMIT NOW! In May 2012, we picked a single leaf from each plant and allowed a randomly chosen \*Spodoptera exigua\* larva to feed on the leaf in a petri dish for 5 days. We used wet body mass at the end of 5 days as our measure of larva performance and consequently plant quality (i.e. small larva = low quality plant tissue).

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).

I WILL ALSO COLLECT LEAVES TO EXAMINE C:N CONTENT ACROSS ALL MY PLANTS.

*Arthropod Food Web*

We quantified arthropod food webs in \_\_\_\_ ways: (1) herbivore and predator/parasitoid abundance, species richness, and rarefied richness, (2) genotype-herbivore network dissimilarity, (3) herbivore-parasitoid network dissimilarity, (4) arthropod community dissimilarity, and (5) average? food chain length.

To do this, we visually surveyed plants for arthropods and enumerated the abundances of each morphospecies on four different occassions from July 2012 - 2013. We created the links within the food web in two ways. For parasitoids, we collected leaf mining, leaf tiering and galling insects and reared them to determine whether they were parasitized. [MAKE A NOTE OF THE RELATIVE ABUNDANCE OF THESE COMPARED TO OTHER HERBIVORES]. For spiders and ants, we created links if they co-occured with potential prey on a given survey. We specified potential prey based on our observations from our visual surveys as well as searching the primary literature. [MAY ONLY USE THE HOST-PARASITOID DATA FOR THE NETWORK STUFF, BUT THE SPIDER AND ANT INFO COULD BE USEFUL FOR THE FOOD CHAIN LENGTH DATA].

*Plant trait variables*

* willow height
* SLA
* Trichome density
* Water content

*Soil variables*

* water
* for wind experiment only
  + nutrients
  + organic matter content

*Community Response Variables*

* Total arthropods
  + Abundance, richness
* Total predators
  + Abundance, richness
  + Spider abundance (dominant group of predators)
* Total herbivores
  + Abundance, richness
  + Leaf rollers and miners (dominant group of herbivores?)
* waiting on belowground community data
* Community Response ~ Environment(s) + (Environment(s) | Genotype) + (1 | Block)

To partition variance explained by each component:

* Community Response ~ (1|Environment(s)) + (1|Genotype) + (1|)

*Details from Lanphere Abiotic/Biotic treatments*

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. The willow, \*Salix hookeriana\*, naturally occurs in dune swales. The dominant vegetation in the swales consistst of \*S. hookeriana\* and beach pine (NEED SPECIES NAME).

### Experimental Design

\*Propogation of willow cuttings\*

Prior to bud burst in February 2012, we took cuttings from one replicate of each of the 10 genotypes used in this experiment that were taken from another common garden where we have replicates of 26 genetically distinct willow clones.

We chose these 10 genotypes because they vary significantly in carbon-nitrogen ratios, LIST OTHER TRAITS

On February 21, 2012 (double check date) we sacrificed 1-2 replicates from 10 of 27 genotypes that varied significantly in leaf carbon:nitrogen ratios (NEED STATS), a putative trait that influences aphid performance. COMMON GARDEN DETAILS. We took cuttings from shoots grown in the previous year and soaked cuttings in water overnight. On February 22, 2012 we planted cuttings in a mixture of 80% perlite, 20% peat moss and \_\_\_ dolomite lime. We grew cuttings under ambient weather conditions outside the greenhouse at Humboldt State University. (do I need to mention that they were exposed to herbivory?)

From May 20-22, 2012 we planted willow cuttings in the swales at the Lanphere Dunes Unit within Humboldt Bay National Wildlife Refuge in California (GPS). We removed all arthropods on the willows at the time of planting. On May 22, we collected aphids (GET SPECIES or GENUS name) 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. 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.

\*Wind Experiment Setup\*

We planted willow cuttings in a completely randomized block experimental design. We established two sites at each of 10 different willow patches (10 blocks). Sites were either exposed or unexposed to the wind (fixed effects) and we planted one replicate of each of 10 genotypes (random effects) in each site (20 treatments \* 10 blocks = 200 total cuttings). We planted exposed and unexposed sites within each block the same distance from the willow patch to control for insect accessability; however, exposed sites faced prevailing winds during the growing season. These wind exposed sites were (GIVE DETAILS ON WIND EXPOSURE).

\*Ant Experiment Setup\*

TO DO

We planted cuttings around 5 different ant mounds (experimental blocks). We planted 20 cuttings (aphid and control treatment of each of 10 genotypes) at each distance of 1.5, 6.5 and 12.5 meters from the edge of the ant mound, for a total of 60 cuttings per ant mound. At each distance, we staggered cuttings at a distance of 25 cm around each distance measurement and ensured that all cuttings were spaced 50 cm apart from each other. Up until May 27, we supplemented planted cuttings with water to promote the survival of cuttings.

To assess the independent effects of willow genotype on aphid performance, we conducted a separate experiment (IN SOLARIUM OR GROWTH CHAMBERS) where we placed 2 adult apterate aphids on the apical shoots of willow cuttings on \_\_\_\_. 2 weeks later, we surveyed cuttings for aphid abundance and used Agrawal 2004 to calculate aphid population growth. We used the mean aphid population growth for each genotype as a continuous explanatory variable of genotype’s effect on ant-aphid interactions.

TO DO

To assess the effects of willow genotype and distance from ant mound on ant-aphid interactions, we surveyed all cuttings June 10, 24, and July 8 and 22 for aphid and ant abundance.

TO DO

To assess the effects of willow genotype and ant-aphid interactions on ecological networks, we surveyed all cuttings on June 24 and July 22 for the abundance and diversity of arthropods. For web-building spiders, we collected their webs and identified the abundance and diversity of their prey items. For herbivore larva, we collected them, brought them into the lab, and fed them leaves until they pupated. We reared pupa until either the original herbivore or an adult parasitoid emerged.

TO DO

To assess the effects of ant-aphid interactions on ecological networks, we also surveyed willow patches that occurred in proximity to thatch-ant mounds. We found \_\_ ant mounds and recorded its distance from the closest willow patch. We recorded whether or not ant-aphid was occurring, and if so, which ant and aphid species were involved (think about recording specific details of ant and aphid abundances…).

TO DO

To assess the effects of willow genotype and ant-aphid interactions on willow performance, we surveyed all cuttings on July 22 for percent leaf area damaged on the 5 most recent leaves of the most apical growing shoot. We scored leaves (0-10 damage score). In addition, we removed all growth from the current year, dried and weighed it to the nearest gram to assess total biomass production from that year.

TO DO

To assess the putative mechanisms by which willow genotype and ant-aphid interactions influence ecological networks, we quantified several traits for each willow cutting, including: specific leaf area (SLA), leaf area, water content, leaf toughness, trichome density, and above ground biomass (CURRENT YEAR SHOOT PRODUCTION OR DRY AND WEIGHT ENTIRE CUTTING?). (GIVE DETAILS IN APPENDIX FOR QUANTIFYING EACH PLANT TRAIT). NEED TO DETERMINE HOW COST-EFFECTIVE IT WOULD BE TO CALCULATE C:N RATIOS OR DEFENSIVE CHEMISTRY FOR ALL PLANTS.

\*Measuring Plant Traits\*

We measured 6 different plant traits at the time herbivores were collected, including: specific leaf area (SLA), leaf size (LS), browned portion of leaf (likely in response to desiccation), plant height, total shoot length, and number of leaves.

\*Soil Measurements\*

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\*Measuring Herbivore Community\*

*Details from ant-aphid manuscript back in the day*

*Experimental Design*

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*Effect of willow genotype and distance from ant mound on ant-aphid interactions*

*Effect of willow genotype and ant-aphid interactions on willow fitness*

*Effect of willow genotype and ant-aphid interactions on host-parasitoid or food web networks*

*Effect of ant-aphid interactions on host-parasitoid or food web networks*

**Field Experiment: Genotype by Ant-Aphid Interactions effect on insect communities**

Completely Randomized Block Experimental Design

* 6 treatments
  + Distance from ant mound: 1, 6, and 12 meters
  + Aphids presence: no addition or add 5 wingless individuals
    - Check that no new aphids have colonized “no addition” plants approximately once a week.
* 10 genotypes (60 cm cuttings)
  + Genotypes vary significantly in C:N ratios which may influence aphid performance.
  + More plant traits will be measured that likely exhibit heritable genetic variation.
* 5 blocks
  + 5 different ant mounds.
* Experimental unit: 1 willow cutting planted into the ground
* Summary of material needed
  + 300 cuttings (5 blocks \* 6 treatments \* 10 genotypes)
  + 750 aphids (5 blocks \* 3 treatments \* 5 aphids per cutting \* 10 genotypes)
* Bag all cuttings to prevent insect colonization. Allow aphids to establish for 24-48 hrs then unbag all plants.
* Survey cuttings approximately once a week to assess ant activity, aphid population growth and aphid parasitism.
* After 2-3 months, survey cuttings for insect abundance and diversity. Sample galls and leaf miners and rear them for their parasitoids.
* Before ending experiment, place one western tent caterpillar larva on each cutting as a bioassay of the effects of ant-aphid interactions on herbivory and survival of non-aphid insects (monitor herbivory and survival every 12-24 hrs).

**Greenhouse Study:** **Plant Traits and Aphid Performance**

- Completely Randomized Experimental Design

* Unbiased assessment of whether there is significant variation among genotypes in plant quality for aphids.
* 2 Treatments
  + Aphids and no aphids
  + 4 replicates of all 10 genotypes per treatment (80 cuttings total from 40 cm trees)
* 200 aphids
* Bag 5 aphids on each aphid treatment cutting and monitor aphid population growth
* Monitor aphid population growth approximately once per week.
* After 4 weeks, measure plant traits of both aphid and non-aphid grown cuttings.
  + Assess constitutive and induced plant traits

**Observational Study:**

* Assess gall and leaf miner densities on branches or trees with and without ants at various distances from thatch-ant mounds
* Rear subsample of galls and leaf miners to assess parasitism.

Results

Discussion

Acknowledgements

References

Figure Legends

Tables

Table 1: Plant traits wind experiment

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 2012 | | | 2013 | | |
| Response | Treatment | df | F | P | df | F | P |
| Survival | Genotype |  |  |  |  |  |  |
|  | Wind exposure |  |  |  |  |  |  |
| Height | Genotype |  |  |  |  |  |  |
|  | Wind exposure |  |  |  |  |  |  |
| Shoot growth | Genotype |  |  |  |  |  |  |
|  | Wind exposure |  |  |  |  |  |  |
| SLA | Genotype |  |  |  |  |  |  |
|  | Wind exposure |  |  |  |  |  |  |
| Leaf water Content | Genotype |  |  |  |  |  |  |
|  | Wind exposure |  |  |  |  |  |  |
| Leaf trichome density | Genotype |  |  |  |  |  |  |
|  | Wind exposure |  |  |  |  |  |  |
| Leaf C:N | Genotype |  |  |  |  |  |  |
|  | Wind exposure |  |  |  |  |  |  |
| Spodoptera performance | Genotype |  |  |  |  |  |  |
|  | Wind exposure |  |  |  |  |  |  |

Table 1: Plant traits ant-aphid experiment

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | 2012 | | | 2013 | | |
| Response | Treatment | df | F | P | df | F | P |
| Survival | Genotype |  |  |  |  |  |  |
|  | Aphids |  |  |  |  |  |  |
|  | Distance to ant mount |  |  |  |  |  |  |
| Height | Genotype |  |  |  |  |  |  |
|  | Aphids |  |  |  |  |  |  |
|  | Distance to ant mount |  |  |  |  |  |  |
| Shoot growth | Genotype |  |  |  |  |  |  |
|  | Aphids |  |  |  |  |  |  |
|  | Distance to ant mount |  |  |  |  |  |  |
| Leaf water Content | Genotype |  |  |  |  |  |  |
|  | Aphids |  |  |  |  |  |  |
|  | Distance to ant mount |  |  |  |  |  |  |
| Leaf trichome density | Genotype |  |  |  |  |  |  |
|  | Aphids |  |  |  |  |  |  |
|  | Distance to ant mount |  |  |  |  |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Response | Predictor | d.f. | F | P |
| Soil |  |  |  |  |
|  |  |  |  |  |
| Survival |  |  |  |  |
|  |  |  |  |  |
| Plant Architecture |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
| Leaf quality |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
| Arthropod community |  |  |  |  |
|  |  |  |  |  |
| Herbivory |  |  |  |  |
|  |  |  |  |  |
| Fungal community |  |  |  |  |

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.