Importance of genotypic variation in host-plants depends on the phenotype and community response being studied

Relative importance of host-plant genotype vs. the environment depends on the environmental stressor

Host-plant genotype predicts phenotype and associated communities across biotic and abiotic environments

Host-plant genotype predicts phenotype and associated communities across diverse environments

Host-plant genotype predicts individual and community phenotypes across diverse environments

Host-plant genotype predicts individual and community responses across diverse environments

Host-plant genotype predicts individual and community responses in spite of abiotic and biotic stressors

Host-plant genotype predicts individual and community responses in spite of environmental stressors

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**Abstract**

**Introduction**

Plant genetic effects can have important effects on plant phenotypes (cite) and associated communities (Whitham et al. 2012).

The majority of studies that have examined the relative importance of plant genotypes vs. the environment have focused on abiotic stressors (cite studies).

To date, there have be numerous studies that have examined the relative importance of plant genotype vs. the environment in determining plant phenotypes and associated communities. These studies have resulted in a diversity of answers, with plant genotype explaining little of the variation (work by Ayco Tack) to genotype being the dominant driver of host-plant variation.

The relative importance of plant genotype vs. the environment appears to be scale-dependent (Johnson and Agrawal, 2005).

A plant’s environment is composed of at least three components, competitive/facilitative interactions with neighboring plants, abiotic environment, and herbivory

The majority of studies

Often, these environmental manipulations may be unrealistic

Some of these environmental manipulations are generic, manipulation soil N

While plant genotype effects have documented their importance, there is still an important call for research addressing the relative importance of plant genotype vs. the environment in shaping phenotypes and resulting community responses.

- Review GxE studies done at various scales? Mostly on a single arthropod species or community?

Here, we address this knowledge gap by examining the relative importance of willow genotype vs. the environment of a coastal dune ecosystem in shaping willow phenotypes and the associated arthropod community. Based on prior work (Barbour et al. 2015), we expected that plant architecture would be more influenced by the environment than leaf quality, due to its tendency to have lower heritability.

We sought to address how do plant genotype and the environment affect plant phenotypes and associated communities? Which plant phenotypes are associated with community responses?

Predicting phenotype and community responses from common garden experiment.

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*).

During preliminary surveys, we qualitatively identified two important sources of environmental variation for willows in the dune swale habitat – wind exposure and aphid herbivory. Willows growing in wind-exposed habitats often exhibit reduced growth, especially at the their leading edge, appearing to be “swept back” by the wind (Fig. 1a). In prior work, we showed that abundance of several herbivore species and guilds was positively associated with willow size (Barbour et al. 2015). Therefore, we hypothesized that willows growing in wind-exposed areas would exhibit reduced growth, which in turn would result in lower arthropod richness and abundance.

For willows in this coastal dune ecosystem, the aphid *Aphis farinosa* was an abundant herbivore in 2012. *Aphis farinosa* is usually found at the tips of new shoot growth where they feed on willow phloem. Phloem is high in sugars, but low in proteins, so aphids have to ingest large volumes of phloem to get a balanced diet (cite). As a result, aphids excrete carbohydrate-rich honeydew, which attracts ants that tend the aphids and feed on the honeydew (citation). This ant-aphid interaction is often mutualistic, because the ants will defend aphids from predatory arthropods (citation). The ant species we observed most frequently tending *A. farinosa* was the western thatching ant, *Formica obscuripes* (Fig. 1b). *F. obscuripes* colonies create distinct dome-shaped mounds from nearby plant-material. Since ants will defend aphids from predatory arthropods, we predicted that both *A. farinosa* and *F. obscuripes* would reach higher abundances on willows growing near mounds of *F. obscuripes*. The abundance of *A. farinosa* and *F. obscuripes* could affect the associated arthropod community in several ways. In the absence of *F. obscuripes*, aphids could either inhibit or facilitate the colonization of other herbivore species through a variety of mechanisms, but we would always expect aphids to attract predatory arthropods. However, since *F. obscuripes* is a large generalist predator, we would expect its presence to reduce the abundance of herbivores and other predators.

*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 plant-growth and leaf traits (Barbour et al. 2015). Shoot cuttings were soaked in water overnight and then planted in a mixture of 80% perlite, 20% peat moss (dolomite lime added to balance pH) 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 use a hand-held anemometer (Kestrel 1000) to measure wind speed at a height of 37 cm aboveground (approximate height of tallest plants in the garden in 2012) in each plot of our experiment. Maximum wind speed measurements were taken over a 30 s period and haphazardly collected on either an exposed or unexposed site first.

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-growth traits – To quantify plant-growth traits, we measured plant height, the number of shoots produced, and average shoot length in late July of each year (end of growing season). We quantified plant height as the distance (mm) from the ground to the tip of the tallest shoot. We quantified average shoot length by measuring every shoot on each plant to the nearest millimeter and calculating the average shoot length for each plant.

Leaf traits – We measured several traits that could shape leaf quality for herbivores, including leaf area, specific leaf area (SLA), water content, trichome density, percentage carbon (C) and nitrogen (N), and C:N. To measure these traits, we excised fully expanded and undamaged leaves from plants in late July of each year, stored leaf samples with a moist paper towel in separate plastic bags within a cooler and immediately brought them back to the laboratory. We then weighed leaves to obtain fresh mass (g), digitally scanned them to measure leaf area (mm2) using ImageJ (Abrámoff, Magalhães, and Ram 2004), and oven-dried them at 60 °C for 72 h to obtain dry weight (g) (Cornelissen et al. 2003). We calculated SLA as (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 center of the leaf, halfway between the leaf edge and the mid-vein, under a dissecting scope. To measure percentage C and N, we ground oven-dried leaves to a fine powder using a ball mill (Mixer/Mill 8000D, SPEX SamplePrep; Metuchen, NJ, USA). Subsamples of each material were then analyzed for percentage C and N on an elemental analyzer (ECS 4010; Costech Analytical Technologies, Valencia, California, USA) using atropine (4.84% N and 70.56% C) as a reference standard.

*Surveying Associated Communities*

Arthropod community – We visually surveyed plants for arthropods to determine the abundances of different (morpho)species. 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. So that individuals were not counted twice between sampling dates, we took the maximum abundance for each arthropod (morpho)species from each plant across all sampling dates within each year. This approach provides a conservative estimate of the total number of individuals of each (morpho)species that occurred on individual plants through the summer. 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.

Mycorrhizal and Bacterial communities – In late July of 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 stored the root tissue of each plant in separate plastic bags within a cooler which were immediately transported back to the lab and kept in a freezer at \_°C until further processing. *(need further details on quantifying the belowground communities from Sonya).*

*Soil characteristics*

Soil nutrients, total organic matter, and moisture may all influence plant phenotypes and 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, percent organic matter, and moisture within each plot of the wind experiment (one exposed and one unexposed plot per block).

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 plot 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 NO3+, NH4-, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, and Cd. To measure percent organic matter content (%OM), 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 plot on three different days between 1100 – 1500 hours. We repeated this same sampling scheme in early July 2013. Plot levels measurements of soil moisture were highly correlated between years (*stats*), so we averaged these soil moisture estimates to determine a single soil moisture value per plot.

*Statistical Analyses*

We used generalized linear mixed-effect models to examine how willow genotype and environmental factors influenced willow phenotypes and univariate community responses (e.g. total abundance, total richness, guild abundance). For the wind experiment, we specified block (willow patch) and plots nested within block (2 wind exposure treatments) as random effects. We specified willow genotype, wind treatment, sampling year, and their 3-way interaction as fixed effects in the model. For some willow phenotypes and guild abundances we did not have data for both years so we removed sampling year from those models. For the ant-aphid experiment, we specified block (ant mound) and plots nested within block (3 different distances from ant mound) as random effects. We specified willow genotype, aphid treatment, distance from ant mound, and their 3-way interaction as fixed effects in the model. For both experiments, we used ‘sum contrasts’ and Type III sum-of-squares in order to reliably test the significance of both main effects and interactions between fixed effects in the full model. For continuous responses (plant height, average shoot length, leaf water content, leaf area, SLA, and leaf C:N) we specified Gaussian error distributions in our models and tested the significance of fixed effects using F-tests with Kenward-Roger approximated degrees of freedom (cite). For count responses (shoot count, trichome density, arthropod richness, and abundance) we specified Poisson error distributions in our models and tested the significance of fixed effects using Wald Chi-square tests. If necessary, we modeled overdispersion in these Poisson models by specifying an individual-level random effect (cite).

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).

Calculating Effect Sizes — We used a recently developed method for calculating *R*2 in mixed-effect models (Nakagawa and Schielzeth 2013) to estimate the effect sizes of willow genotype and environmental factors on willow phenotypes and community responses. To do this, we fit reduced models with only the significant fixed effects included. If willow genotype and/or its higher-order interactions with the environment were significant, we refit them as random effects. We re-specified genotype as a random effect because preliminary analyses found that treating it as a fixed effect overestimated the variance explained. This is likely because *R*2is estimated without degrees-of-freedom (*df*) correction (Nakagawa and Schielzeth 2013). For example, when willow genotype is specified as a fixed effect it has *df* = 9 whereas each of our environmental factors has *df* = 1, which inherently bias genotype towards a higher amount of variance explained even if it is not significant as a fixed effect. An alternative approach that has been advocated to calculate effect sizes for genotype-by-environment experiments is to treat both genotype and environment factors as random effects (Hersch-Green et al. 2012). However, experimental manipulations of the environment often have a smaller number of levels (e.g. ‘exposed’ and ‘unexposed’ wind treatments) and it is know that mixed-effect models underestimate the variance explained by random effects with a small number of levels (i.e. < 5). Therefore, we feel this hybrid-approach (environment as a fixed effect and genotype as a random effect) is a robust approach for comparing the relative importance of genotype vs. the environment in our study.

**Results**

*Wind experiment*

Willows growing in wind-exposed plots experienced up to 3.7-fold higher wind speeds compared to unexposed plots (F1,9 = 187.32, P < 0.001). Despite these large differences in wind exposure, wind-exposure had modest effects on soil properties. Specifically, soil in wind-exposed plots tended to be drier (F1,9 = 3.52, P = 0.093) with higher amounts of total Nitrogen (F1,9 = 5.08, P = 0.051). There was no difference in percent organic matter (F1,8.4 = 0.68, P = 0.434) or nutrient composition (PC1: F1,9 = 1.31, P = 0.282) between soils in wind-exposed and unexposed plots. Although most of these soil properties were highly correlated (supplementary table), only soil moisture had a detectable effect on plant traits. Soil moisture was positively correlated with plant height (r = 0.58, t18 = 3.03, P = 0.007), shoot count (r = 0.46, t18 = 2.18, P = 0.043), but negatively correlated with leaf water content (r = -0.58, t18 = -2.99, P = 0.008), so we included it as a covariate in our experimental analyses of these traits.

Phenotypes:We found that plant-growth traits were influenced by wind exposure, sampling year, and willow genotype (Table 1). Wind exposure negatively affected all plant-growth traits, resulting in willows that were 23% shorter in height (*R2* = 0.07), produced 22% fewer shoots (*R2* = 0.05), and grew 28% shorter shoots (*R2* = 0.04) compared to willows in plots protected from the wind. The negative effects of wind on plant growth traits were primarily due to direct effects of wind pruning (height std. = -0.28; shoot count std. = -0.21) rather than an indirect effect mediated by reduced soil moisture (height std. = -0.08; shoot count std. = -0.05)*.* For both plant height and the number of shoots, the negative effects of wind exposure were magnified in 2013 compared to 2012 (plant height, EWINDEYEAR *R2* = 0.02; shoot count, EWINDEYEAR *R2* = 0.02). Sampling year had a large effect on plant-growth traits; by the second year of the experiment, willows were 39% shorter in height (*R2* = 0.24) and grew 43% shorter shoots (*R2* = 0.13). Willow genotype also had a pronounced effect on all plant-growth traits, resulting in willows that varied 2-fold in height (*H2* = 0.12), 2.3-fold in number of shoots (*H2* = 0.11), and 2.2-fold in shoot length (*H2* = 0.07) among the most disparate genotypes. Although willow genotype explained more of the variance in plant-growth traits than wind exposure, the aggregate effects of the environment (Ewind + Eyear + EwindEyear) were often more important (plant height: environment *R2* = 0.35; shoot count: environment *R2* = 0.10; shoot length: environment *R2* = 0.17).

In contrast to plant-growth traits, willow genotype was the primary factor in determining leaf traits across both years of the experiment (Table 1). The leaves of willow genotypes varied 46-fold in trichome density (*H2* = 0.65), 1.5-fold in SLA (*H2* = 0.25), and 1.6-fold in C:N (*H2* = 0.25). We had data available on leaf water content for 2012 and 2013, and we found that the broad-sense heritability of leaf water content depended on the sampling year (2012, *H2* = 0.11; 2013, *H2* = 0.16).

Communities: The responses of the arthropod community mirrored those of plant-growth traits, depending on wind exposure, sampling year, and willow genotype (Table 1). The composition of the arthropod community differed between wind-exposed and unexposed willows, with wind-exposed willows having higher relative abundances of aphids and leaf-tiering moths. This was because aphids and leaf-tiering moths were insensitive to wind exposure, while other key arthropod taxa (gall midges, leaf mining moths, and spiders) were much less abundant on wind-exposed willows (Table 1). The negative response of these key arthropods to wind exposure resulted in 51% fewer species (*R2* = 0.10) and 47% fewer individuals (*R2* = 0.03) on willows in wind-exposed plots. The negative effect of wind exposure on arthropod richness was not solely due to lower arthropod abundance, as rarefied richness was also 60% less on wind-exposed willows (*R2* = 0.20). Overall, these negative effects of wind exposure were primarily mediated by an indirect effect on plant height (abundance, std. = ; richness, std. = ) rather than a direct effect of being exposed to a more windy environment (abundance, std. = ; richness, std. = ).

Willow genotype also had a strong effect on the arthropod community, with the majority of the key taxa varying in abundance among willow genotypes (Table 1), but with little coherence in their responses (i.e. no genetic correlation, Table \_). Still, willow genotype influenced arthropod richness and abundance, with arthropods varying 3.1-fold in richness (*R2* = 0.04) and 4.7-fold in abundance (*R2* = 0.06) among the most disparate willow genotypes (Table 1). Unlike wind exposure, the effect of willow genotype on arthropod richness appeared to be solely due to abundance, as rarefied richness did not differ among willow genotypes (Table 1).

In contrast to the arthropod community, the richness and abundance of root-associated fungi and bacteria did not appear to be influenced by wind exposure or willow genotype (Table 1).

*Ant-aphid experiment*

Phenotypes:In contrast to the wind experiment, we found that both plant-growth and leaf traits were influenced solely by willow genotype in the ant-aphid experiment (Table 2). For example, willow genotypes varied 2-fold in plant height (*H2* = 0.27) and 30-fold in leaf trichome density (*H2* = 0.76). We also observed strong effects of willow genotype on the number of shoots produced (*H2* = 0.16) and shoot length (*H2* = 0.15), but there was little apparent effect of willow genotype on leaf water content in this experiment (Table 2).

Communities: In contrast to the plant traits, the responses of the arthropod community were contingent on the presence of aphids, distance from mounds of *F. obscuripes*, and willow genotype (Table 2). In the presence of *A. arinose*, the abundance of other arthropods increased 1.8-fold on willows that were further from mounds of *F. obscuripes*, an effect that was primarily driven by leaf-mining herbivores (Family: Gracilliaridae). The presence of *A. arinose* also modified the effect of willow genotype on arthropod abundance, an effect that was primarily due to the response of other aphids. In spite of these biotic interactions, willow genotype still had a strong effect on the richness and abundance of arthropods, which varied 2.6- and 4-fold, respectively, among the most disparate willow genotypes. The effect of willow genotype on arthropod richness appeared to be due to correlated responses in arthropod abundance, as willow genotype had no effect on the rarefied richness of arthropods.

*Predicting Phenotype and Arthropod Community Responses.*

**Discussion**

Key Findings:

- Biotic interactions had little effect on willow phenotypes, but directly affected the willow’s associated arthropod community. Still, genotype was the primary determinant of variation in its phenotype and its associated community.

- Abiotic interactions had strong effect on willow growth traits, which had strong indirect effects on the richness, abundance, and composition of the arthropod community. However, genotype was still comparable in its effects on willow growth traits and its associated arthropod community. However, the traits we measured were insufficient to explain the effect of willow genotype on the arthropod community. This result has repeated itself in previous work in this system (Barbour et al. 2015). To date, the functional trait approach (quantifying easy to measure traits) is failing in its ability to predict arthropod community responses. How do we improve this? I think it will involve careful consideration of which plant traits arthropods are responding too. We also, don’t really have a good sense for how much of the variation we should expect traits to explain. Is it all of it? Perhaps we need to start incorporating stochasticity into arthropod community ecology. This may give us a more realistic benchmark for how much variation we expect to be explained by plant traits.

Acknowledgements

References

Figure Legends

**Tables**

**Table 1**:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Response | Genotype (G) | Ewind | GEwind | Eyear | GEyear | EwindEyear | GEwindEyear |
| **Plant-growth traits** |  |  |  |  |  |  |  |
| Plant height | **7.46** | **26.87** | 0.79 | **183.73** | 1.05 | **9.70** | 1.62 |
| Shoot count | **54.02** | **11.74** | 11.58 | 2.39 | 15.12 | **9.91** | 5.78 |
| Shoot length | **4.70** | **9.86** | 0.80 | **75.07** | 1.43 | 0.11 | 0.70 |
| **Leaf traits** |  |  |  |  |  |  |  |
| Trichome density2012 | **81.35** | 0.11 | 10.77 | - | - | - | - |
| C:N2013 | **4.98** | 0.74 | 1.30 |  |  |  |  |
| Water content | **4.89** | 0.93 | 0.44 | 2.51 | **2.73** | 1.80 | 1.56 |
| SLA2013 | **5.89** | 0.44 | 0.86 | - | - | - | - |
| **Root traits** |  |  |  |  |  |  |  |
| C:N2013 | 0.91 | 0.23 | 0.30 | - | - | - | - |
| **Arthropods** |  |  |  |  |  |  |  |
| Total abundance | **28.63** | **10.96** | 8.08 | 2.25 | 9.88 | **3.90** | 11.54 |
| Total richness | **26.39** | **21.60** | **3.39** | 4.31 | 8.28 | 0.63 | 7.02 |
| Total PIE | 1.65 | **8.74** | 1.61 | 0.04 | - | 1.17 | - |
| Leaf-mining moths (Gracilliaridae) | **17.15** | **9.42** | - | **4.26** | - | 0.09 | - |
| Gall midges (Cecidomyiidae) | **19.26** | **17.59** | - | **38.07** | - | - | - |
| Leaf-tiering moths (Tortricidae) | **24.78** | 1.34 | 11.50 | **117.19** | - | 2.65 | - |
| Aphididae2012 | 4.31 | 0.32 | - | - | - | - | - |
| Spiders | 8.27 | **6.04** | - | **6.54** | - | 0.20 | - |
| **Mychorrizae2013** |  |  |  |  |  |  |  |
| Total abundance | 1.01 | 0.95 | 1.03 |  |  |  |  |
| Total richness | 1.65 | 1.85 | 1.23 |  |  |  |  |
| Community composition | **1.01** | **2.09** | 0.87 |  |  |  |  |
| Phylum composition |  |  |  |  |  |  |  |
| **Bacteria2013** |  |  |  |  |  |  |  |
| Total abundance | 1.25 | 2.18 | 0.64 |  |  |  |  |
| Total richness | 1.30 | *4.17* | 0.87 |  |  |  |  |
| Community composition |  |  |  |  |  |  |  |
| Phylum composition |  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Response** | **Genotype (G)** | **Eaphid** | **Eant** | **GEaphid** | **GEant** | **EaphidEant** | **GEaphidEant** |
| **Plant-growth traits** |  |  |  |  |  |  |  |
| Plant height | **15.39** | 0.19 | 0.38 | 0.93 | 0.95 | 0.16 | 1.62 |
| Shoot count | **60.40** | 0.60 | 0.38 | 10.67 | 8.29 | 2.76 | 9.17 |
| Shoot length | **6.55** | 1.67 | 0.30 | 1.07 | 0.68 | 0.88 | 0.56 |
| **Leaf traits** |  |  |  |  |  |  |  |
| Trichome density | **46.99** | 0.22 | 0.34 | - | 8.79 | 0.43 | - |
| Water content | 1.56 | 0.01 | 0.17 | - | 0.87 | 0.44 | - |
| **Arthropods** |  |  |  |  |  |  |  |
| Total abundance | **43.44** | *3.08* | 0.79 | **18.15** | 10.13 | **7.96** | 0.36 |
| Total richness | **46.74** | 2.49 | 0.68 | 7.67 | 8.91 | 0.86 | 6.60 |
| Total PIE | 1.09 | 1.49 | 2.04 | 1.54 | 0.88 | 0.03 | 0.67 |
| *Aphis farinosa* | **18.17** | - | 1.21 | - | 9.33 | - | - |
| *Formica obscuripes* | R | **7.45** | *3.10* | - | - | - | - |
| Gracilliaridae (leaf mines) | **35.43** | 1.56 | **5.21** | 11.41 | *15.06* | **4.19** | - |
| Tortricidae (leaf tiers) | **24.55** | 0.03 | **11.87** | - | - | *3.65* | - |
| Aphididae (non-*Aphis*) | **30.89** | 0.07 | 0.52 | **19.00** | 5.87 | *3.82* | 6.88 |
| Spiders (predators) | *14.61* | 0.24 | 0.93 | 12.28 | 8.85 | 0.01 | - |
| Formicidae (non-*F. obscuripes*) | **20.65** | **14.71** | 1.29 | 4.89 | 6.76 | 0.73 | - |
| Cicadellidae (sap feeder) | **18.88** | 0.85 | 2.13 | 6.65 | 10.15 | 1.68 | - |

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).