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

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**Abstract**

**Introduction**

Plant genes determine individual phenotypes, which can have cascading effects on associated species and entire communities of organisms (Fritz & Price 1988; Maddox & Root 1990; Antonovics 1992; Lamit *et al.* 2015) . While the importance of plant genes for associated communities is well established in common gardens where environmental variation is minimized, the relative importance of plant genotype vs. the environment still remains an open question (Hersch-Green *et al.* 2011; Tack *et al.* 2012; Crutsinger 2015) . Addressing this question is critical for understanding the dynamic interplay between ecological and evolutionary processes in shaping communities (Johnson & Stinchcombe 2007; Hughes *et al.* 2008; Hersch-Green *et al.* 2011) .

In genotype-by-environment studies, “environment” is often a catch-all term that encompasses a diversity of abiotic and biotic factors. For example, many studies manipulate the environment by planting common gardens in distant locations that likely differ in both abiotic (e.g. soil properties) and biotic (e.g. species pool) factors. This work has provided invaluable insight to the importance of spatial scale (Johnson & Agrawal 2005; Tack *et al.* 2010; Silfver *et al.* 2015); however, it is difficult to tease apart the effects of the many abiotic and biotic factors that could affect community assembly. Studies conducted at smaller spatial scales are advantageous in that they can focus on a single abiotic or biotic factor (Johnson 2008; Abdala‐Roberts *et al.* 2012; Abdala‐Roberts & Mooney 2013); still though, we have a limited understanding of the relative importance of local abiotic and biotic factors in shaping communities associated with host-plants.

While there have been several studies examining the joint contribution of the environment and host-plant genotype to associated communities, the processes generating community responses often remain unclear (Hersch-Green *et al.* 2011; Crutsinger 2015). This is because many studies do not measure the plant phenotypes mediating the interactions between plant genotype and the associated community. Identifying these key phenotypes is also crucial for teasing apart the direct and indirect (via plant traits) effects of the environment on community assembly. Indeed, we should be able to predict which phenotypes are more likely to be influenced by the environment based on prior estimates of heritability. Heritability measures the proportion of variance in a phenotype explained by genotype (Lynch & Walsh 1998); therefore, we expect that traits with low heritability are more likely to be altered by the environment compared to traits that are highly heritable. This can have important consequences for predicting community assembly depending on whether species are cueing in on traits that are weakly or strongly heritable.

Host-plants are usually colonized by a diverse group of organisms, including arthropods, fungi, and bacteria; however, most studies examine the associations between host-plant genotype and a particular taxonomic group (reviewed in Whitham *et al.* 2012; but see Crutsinger 2014; Lamit *et al.* 2015). In particular, the majority of studies have been conducted on aboveground arthropods, with comparatively little attention given to belowground mycorrhizal and bacteria communities. As a consequence, it is unclear whether these diverse communities exhibit similar or different responses to genetic and environmental variation. A recent meta-analysis of the well-studied introgression between *Populus fremontii* and *Populus angustifolia* suggests that aboveground arthropods are more tightly coupled to host-plant genotype than belowground microbes/fungi (Bailey *et al.* 2009). However, there has been little work that has simultaneously examined above- and belowground community responses within genotype-by-environment studies.

Here, we use common garden experiments to examine how host-plant genetics as well as the abiotic and biotic environment structure communities associated with the willow *Salix hookeriana* in a coastal dune ecosystem. Prior work in this system has shown that willow genotypes host distinct arthropod communities and that multiple plant phenotypes are important in determining community assembly (Barbour *et al.* 2015, 2016). Importantly, these phenotypes varied substantially in the degree of heritability (plant growth, mean *H*2 = 0.26; leaf quality, mean *H*2 = 0.72), suggesting that the environment may differentially influence them. We sought to address the following questions: (1) what is the relative importance of willow genotype vs. the abiotic and biotic environment in determining willow phenotypes and associated communities? (2) What are the mechanisms by which willow genetic and environmental variation affects community responses? (3) Do host-associated arthropods, fungi, and bacteria exhibit similar or contrasting responses to willow genetic and environmental variation?

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 dunes – wind exposure and ant-aphid mutualisms. 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). We also observed that the aphid *Aphis farinosa* was an abundant herbivore at Lanphere Dunes 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 and also eat other herbivores that may be competing with the aphids (Floate & Whitham 1994; Mooney & Agrawal 2008). The ant species we observed most frequently tending *A. farinosa* was the western thatching ant, *Formica obscuripes* (Fig. 1b). Thatch ant colonies create distinct dome-shaped mounds from nearby plant-material. The strength of this mutualistic interaction may also decrease at further distances from ant colonies (Wimp & Whitham 2001).

*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 until we transplanted willows into multiple common gardens at Lanphere Dunes.

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 2012. Within each block, we randomly planted 20 cuttings (2 replicates of each of 10 genotypes) with 0.5 m spacing in plots that were at a 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). Within each plot, we randomly assigned the aphid treatment (aphid presence vs. absence) to one of the two replicates for each genotype. 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. 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 water content, trichome density, specific leaf area (SLA), 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 (Erlandson *et al.* 2015). Moreover, we expected that wind exposure to affect these soil characteristics (LORTIE & CUSHMAN 2007); 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. From this nutrient data, we calculated total N as NO3+ + NH4-, and then used principal components analysis to condense these nutrients into a single axis (nutrients PC1) that explained 34% of the variation. Nutrients PC1 described the negative correlation between nitrogen compounds (NO3+, NH4-) and the rest of the ionic elements, with positive values indicating high supply rates of all ionic elements except for the nitrogen compounds. 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 (Pearson’s *r* = 0.93, *t*18 = 10.91, P < 0.001), so we averaged these soil moisture estimates to determine a single soil moisture value per plot.

*Statistical Analyses*

Community responses – We used separate generalized linear mixed-effect models (Bolker *et al.* 2009) to examine how willow genotype, the environment, and their interaction influenced richness, abundance, and rarefied richness of aboveground arthropods as well as root-associated mycorrhiza and bacteria. 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 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 Type II sum-of-squares to test the significance of fixed effects. 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. For count responses (shoot count, trichome density, arthropod richness and abundances) we specified Poisson error distributions in our models and tested the significance of fixed effects using likelihood-ratio tests. If necessary, we modeled overdispersion in these Poisson models by specifying an individual-level random effect.

For the mycorrhizal and bacterial community data, we used separate redundancy analyses (RDA) to examine how willow genotype, wind exposure, and their interaction influenced the proportional abundance of species (i.e. community composition). To do this, we applied a Hellinger transformation to the community data prior to analysis, as this has been shown to reliably characterize the dissimilarity of diverse communities (Legendre & Gallagher 2001). To test the significance of each term, we compared the observed community dissimilarities to the dissimilarities we would expect by random chance with a permutation test that controls for the blocked design of our experiment.

Plant traits and Mechanisms of community assembly –

To examine the effect of wind exposure on maximum wind speed and soil characteristics (total N, nutrients PC1, %OM, and soil moisture), we used separate mixed effect models with wind treatment as a fixed effect and block (willow patch) as a random effect. Since all soil characteristics were continuous responses, 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.

Calculating Effect Sizes — We used a recently developed method for calculating *R*2 in mixed-effect models (Nakagawa & Schielzeth 2013) to estimate the effect sizes of willow genotype and environmental factors on willow phenotypes and community responses. Prior to calculating *R*2, 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 & 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 for calculating effect sizes in genotype-by-environment experiments is to treat both genotype and environment factors as random effects (Hersch-Green *et al.* 2011). However, experimental manipulations of the environment often have a smaller number of levels (e.g. ‘exposed’ and ‘unexposed’ wind treatments) and it is known 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 and likely others.

Predicting the effects of willow genotype —To analyze these data, we analyzed the correlation between mean genotype values for individual and community phenotypes.

All analyses were conducted in R version 3.2.4 (R Core Team 2016). All code and data to replicate these analyses has been deposited in Zenodo (DOI: \_\_\_).

**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, we observed only modest effects of wind exposure 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 aboveground plant traits. Soil moisture was positively correlated with plant height (r = 0.58, t18 = 3.03, P = 0.007) and 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).

Individual phenotypes:We found that plant-growth traits were influenced by wind exposure, sampling year, and willow genotype (Table 1). As expected, wind exposure negatively affected all plant-growth traits. Moreover, the negative effects of wind were magnified by the end of the experiment for both plant height and the number of shoots produced (Table 1). 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; shoot length std. = -0.21) rather than an indirect effect mediated by reduced soil moisture (height std. = -0.08; shoot count std. = -0.05; shoot length std. = -0.02)*.* Still, willow genotype had a pronounced effect on all plant-growth traits, resulting in willows that varied over 2-fold in height, number of shoots, and shoot length among the most disparate genotypes. While the effect of willow genotype on shoot length changed by the end of the experiment (Table 1), this GEyear effect was relatively small (*R2* = 0.05) compared to the effect of genotype alone (*R2* = 0.13). 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, 1.5-fold in SLA, and 1.6-fold in C:N. We had data available on leaf water content for 2012 and 2013, and we found that the amount of variation explained by willow genotype depended on the sampling year (2012, *R2* = 0.11; 2013, *R2* = 0.16). While soil moisture did affect leaf water content (std. = -0.13), the amount of variation it explained was small (*R2* = 0.02) compared to willow genotype. Unlike plant-growth and leaf traits, root C:N did not appear to be influenced by either wind exposure or willow genotype.

Community phenotypes: Wind exposure, sampling year, and willow genotype all had strong, independent effects on the arthropod community (Table 1). Willows growing in wind-exposed plots hosted 51% fewer species, 47% fewer individuals, 60% fewer rarefied species compared to unexposed willows. The negative effects of wind exposure on the arthropod community were due to both direct effects (abundance std. = -0.04; richness std. = -0.19; evenness std. = -0.12) as well as indirect effects mediated by reductions in plant height (abundance std. = -0.05; richness std. = -0.10; evenness std. = -0.14). Arthropod communities on willows had both more species and more individuals in the second year of the experiment compared to the first (Table 1); however, this effect could simply be an artifact of us conducting more arthropod surveys for the wind experiment in 2013 vs. 2012. In spite of the effects of wind exposure and sampling year, willow genotype had a strong effect on both the richness (3.1-fold differences) and abundance (4.7-fold differences) of arthropods, but only a marginal effect on their probability of interspecific encounter. The indirect effect of genotype on arthropod richness was mediated solely through variation in willow height (std. = 0.11), while effects on total abundance were mediated by willow height (std. = 0.05), leaf water content (std. = 0.03) and leaf C:N (std. = 0.04).

We observed strong effects of wind exposure on the composition of the arthropod community by the end of experiment (Table 1). These compositional differences were due to several key arthropod taxa (gall midges, leaf-mining moths, and spiders) being less abundant on wind-exposed willows, whereas leaf-tiering moths were insensitive to wind exposure (and therefore relatively more abundant; Fig. ). In contrast to wind exposure, willow genotype did not appear to affect community composition in either year (Table 1), although several arthropod taxa varied in abundance among willow genotypes (Table 1; Fig. \_).

In contrast to the arthropod community, neither wind exposure nor willow genotype appeared to influence the richness, abundance, or evenness of the root-associated mycorrhiza community (Table 1, Fig. \_). However, willow genotype had a modest independent effect on the composition of the mycorrhizal community (*R*2 = 0.07, Fig. \_), whereas wind-exposure had no detectable effect. In contrast to the mycorrhizal community, we observed small effects of wind exposure on multiple indices of the bacteria community, with no detectable effect of willow genotype (Table 1). For example, the roots of wind-exposed plants tended to host more bacteria OTUs (10% increase) than unexposed plants. Wind-exposed plants also had a more evenly distributed bacteria community, but the effect size was very small (wind-exposed PIE = 0.9993, unexposed PIE = 0.9992) (Table 1). While wind exposure did not affect the total abundance of bacteria OTUs, it had a marginal effect on the the composition of the bacteria community (Fig \_).

*Ant-aphid experiment*

Willow genotype had a strong effect on *Aphis farinosa* abundance ( = 20.83, P = 0.013), whereas mound distance did not influence aphid abundance ( = 0.55, P = 0.460). Both aphid treatment and mound distance independently affected the probability of finding *F. obscuripes* tending *Aphis farinosa.* The probability of finding *F. obscuripes* on willowsincreased from < 1% to 10% in the aphid treatment ( = 28.10, P < 0.001) and decreased from 4% at to < 1% at 12 m from the ant mound ( = 4.02, P = 0.045). Genotype did not appear to have a strong effect on the probability of finding *F. obscuripes* ( = 0.98, P = 0.322).

Individual phenotypes:In contrast to the wind experiment, we found that both plant-growth and leaf traits were influenced primarily by willow genotype rather than the environmental factors in the ant-aphid experiment (Table 2). For example, all of the plant-growth traits we measured varied approximately 2-fold among the most disparate willow genotypes. Willows also appeared to produce slightly more shoots in the absence of aphids and at further distances from ant mounds (Table 2), but this effect was weak (*R*2 = 0.01) compared to willow genotype (*R*2 = 0.15). Similar to the wind experiment, leaf trichome density varied 30-fold among willow genotypes, but there was little apparent effect of willow genotype on leaf water content in the ant-aphid experiment (Table 2).

Community phenotypes: Similar to the plant traits, willow genotype was the primary determinant of arthropod community responses. We found that the richness and abundance of arthropods 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 probability of interspecific encounter. WHICH TRAITS ARE MEDIATING ARTHROPOD RESPONSES. We did detect a significant EaphidEant effect on arthropod abundance (Table 2). Arthropods were 1.8-fold more abundant on aphid-treated willows at 12 m vs. 2 m from ant mounds; however, this EaphidEant effect was small (*R*2 = 0.02) in comparison to the effect of willow genotype (*R*2 = 0.14). In contrast to the independent effects of willow genotype and biotic factors on aggregate community responses, we found a significant willow GEaphid effect on arthropod community composition. This GEaphid effect appeared to be solely due to differences in the abundance of non-*A. farinosa* aphids, which was also primarily determined by the effects of the aphid treatment on a single willow genotype (Fig. \_).

Predicting effects of genotype across experiments – Despite the sometimes important effect of wind and ant-aphid interactions on the individual and community phenotypes of willow, we found strong genetic correlations in the majority of these phenotypes across experiments. We found the following genetic correlations across experiments: plant height (r = 0.88, t9 = 5.30, P < 0.001), leaf water content (r = 0.70, t8 = 2.81, P = 0.023), leaf trichome density (r = 0.89, t8 = 5.55, P < 0.001), leaf-tiering moths (r = 0.74, t8 = 3.10, P = 0.015), aphids (r = 0.65, t8 = 2.43, P = 0.041), except for the leaf-mining moths (r = 0.32, t8 = 0.95, P = 0.370).

**Discussion**

Willow genotype had predictable effects on individual phenotypes and the arthropod community across variation in both the abiotic and biotic environment (FIG or TABLE). These predictable effects occurred despite the abiotic environment often explaining as much or more of the variation in plant-growth traits and arthropod responses.

- Aboveground, we found that wind exposure dominated both plant genotype and ant-aphid interactions in explaining arthropod community responses.

- Belowground, mycorrhizal and microbial communities responded differently than aboveground arthropods to willow genotype and wind exposure.

- Despite the fact that the environment sometimes dominated plant genotype in explaining community responses, the effects of plant genotype were predictable across variation in both the abiotic and biotic environment (Fig. genetic correlations).

Updated Key Findings:

- Arthropod community responses: Abiotic > Genotype > Biotic

- Mycorrhizal community responses: Genotype > Abiotic

- Microbial community responses: Abiotic > Genotype

- Plant-growth traits: Abiotic = Genotype > Biotic

- Leaf-quality traits: Genotype > Abiotic = Biotic

- Host-plant associated communities responded differently to variation in host-plant genotype and the environment.

- Measuring the plant traits enabled us to tease apart the direct and indirect effects of genotype and the environment in determining community responses. Most everything was determined by plant height, with a secondary contribution from other plant traits.

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.

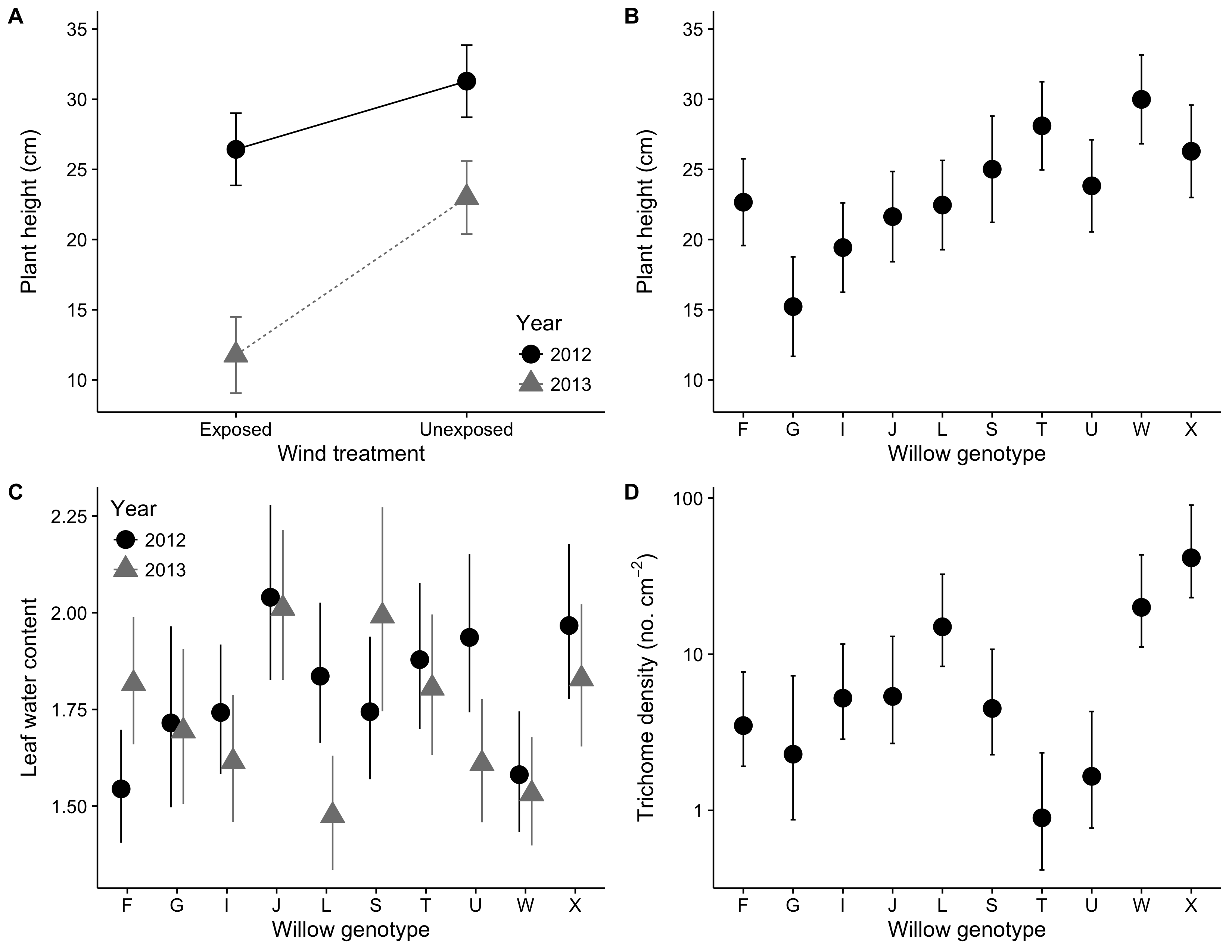
- Abiotic interactions had strong effect on willow growth traits, which had strong indirect effects on the richness, abundance, and composition of the arthropod community. However, genotype was still comparable in its effects on willow growth traits and its associated arthropod community. However, the 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.

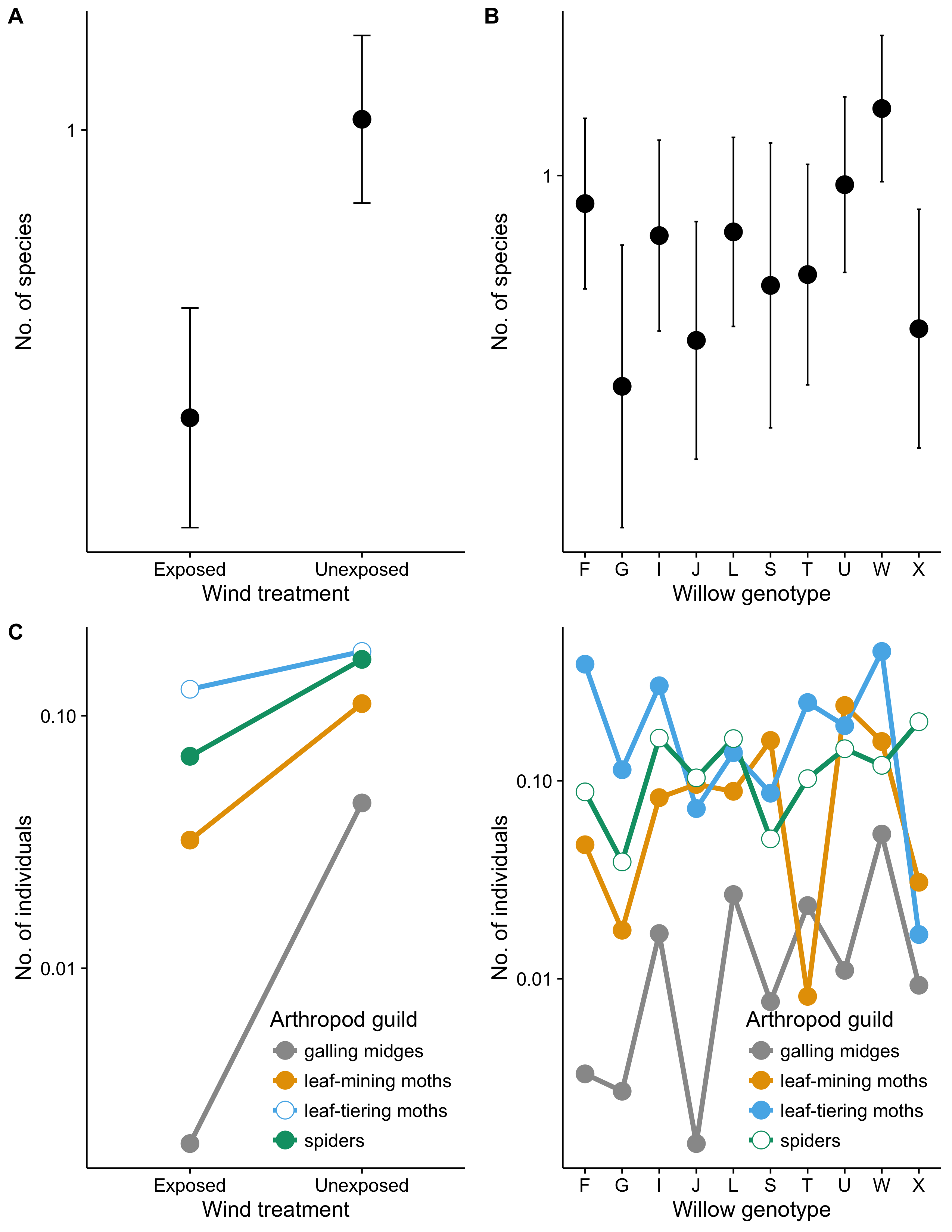
- Genotypes still had predictive power on individual and community phenotypes across both experiments.

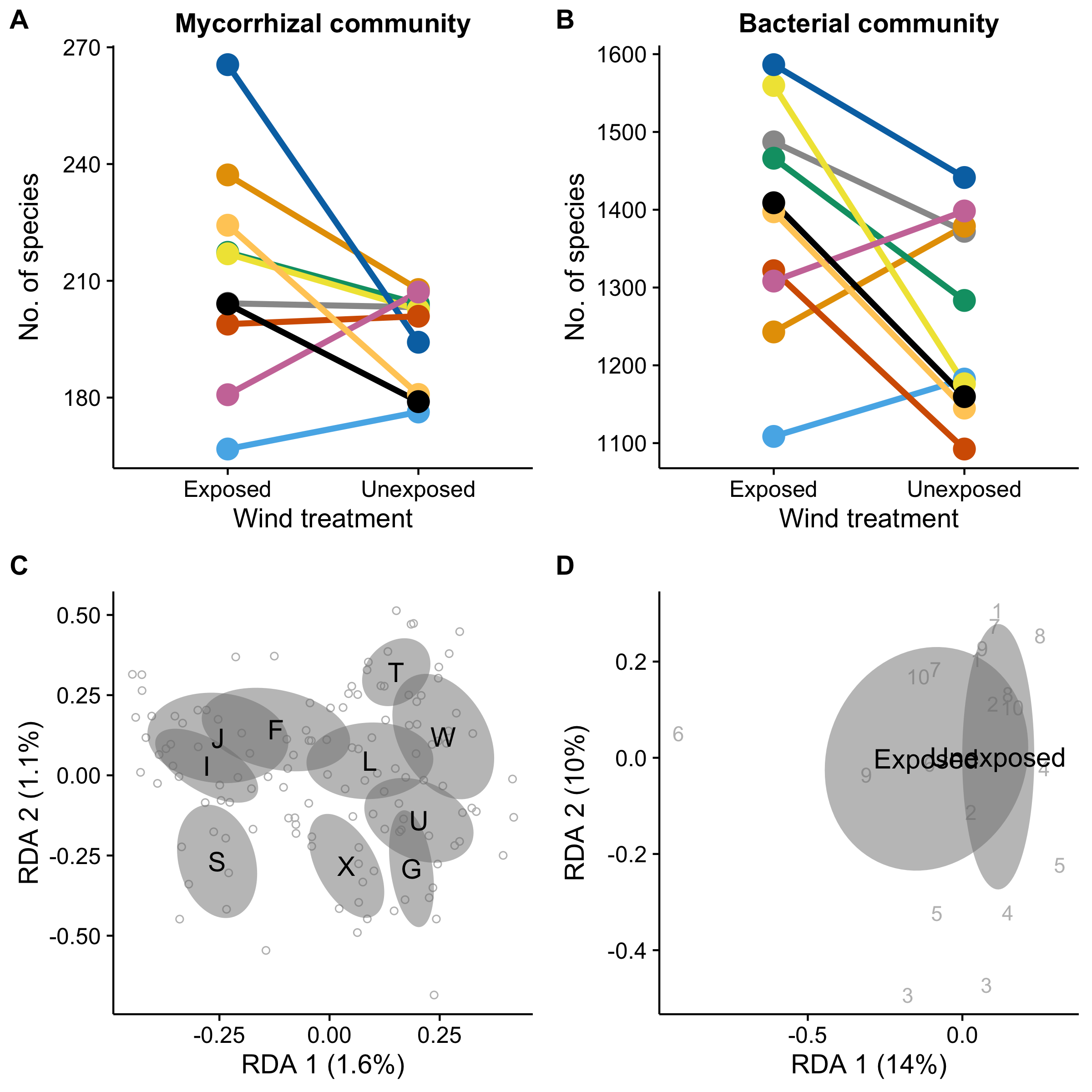
**Acknowledgements**

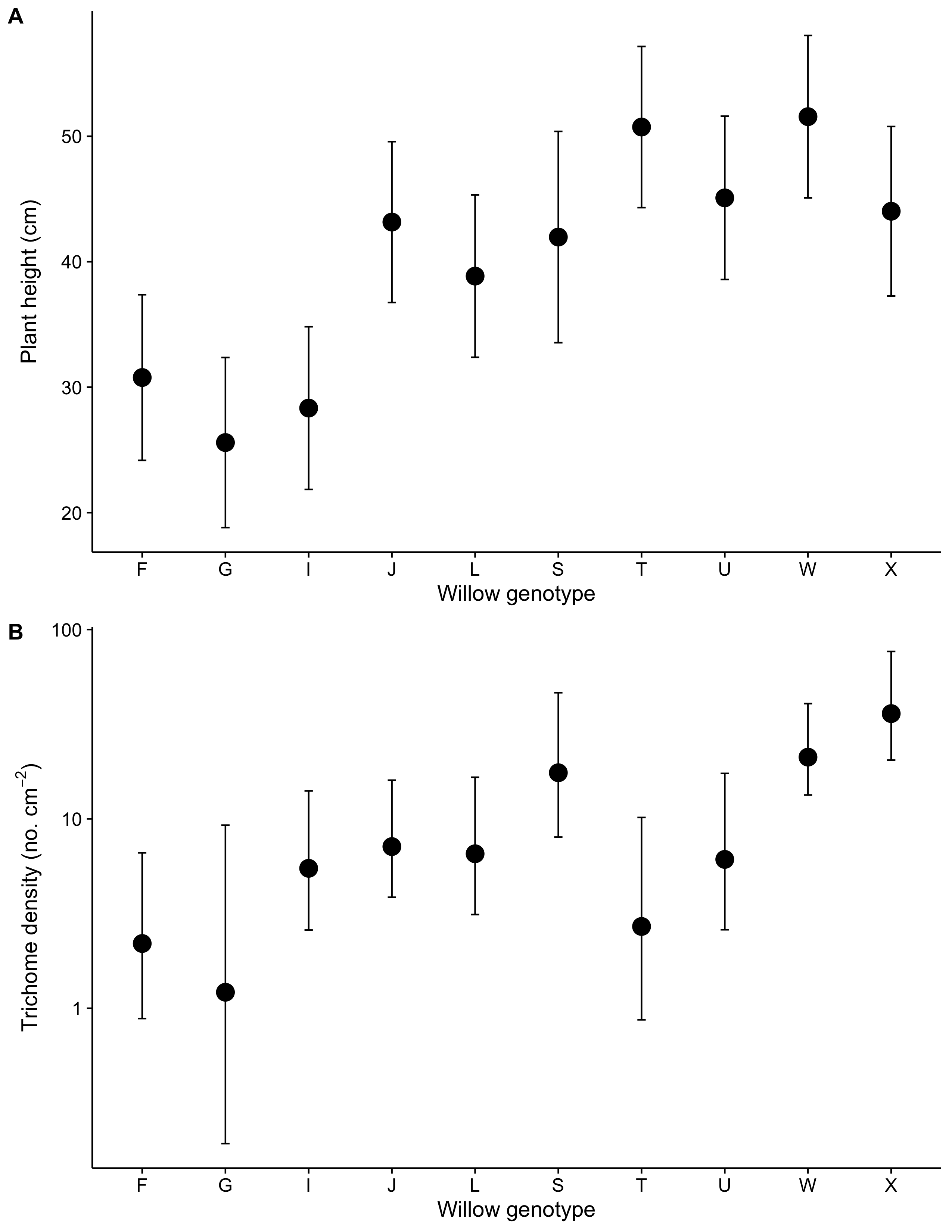
**References**

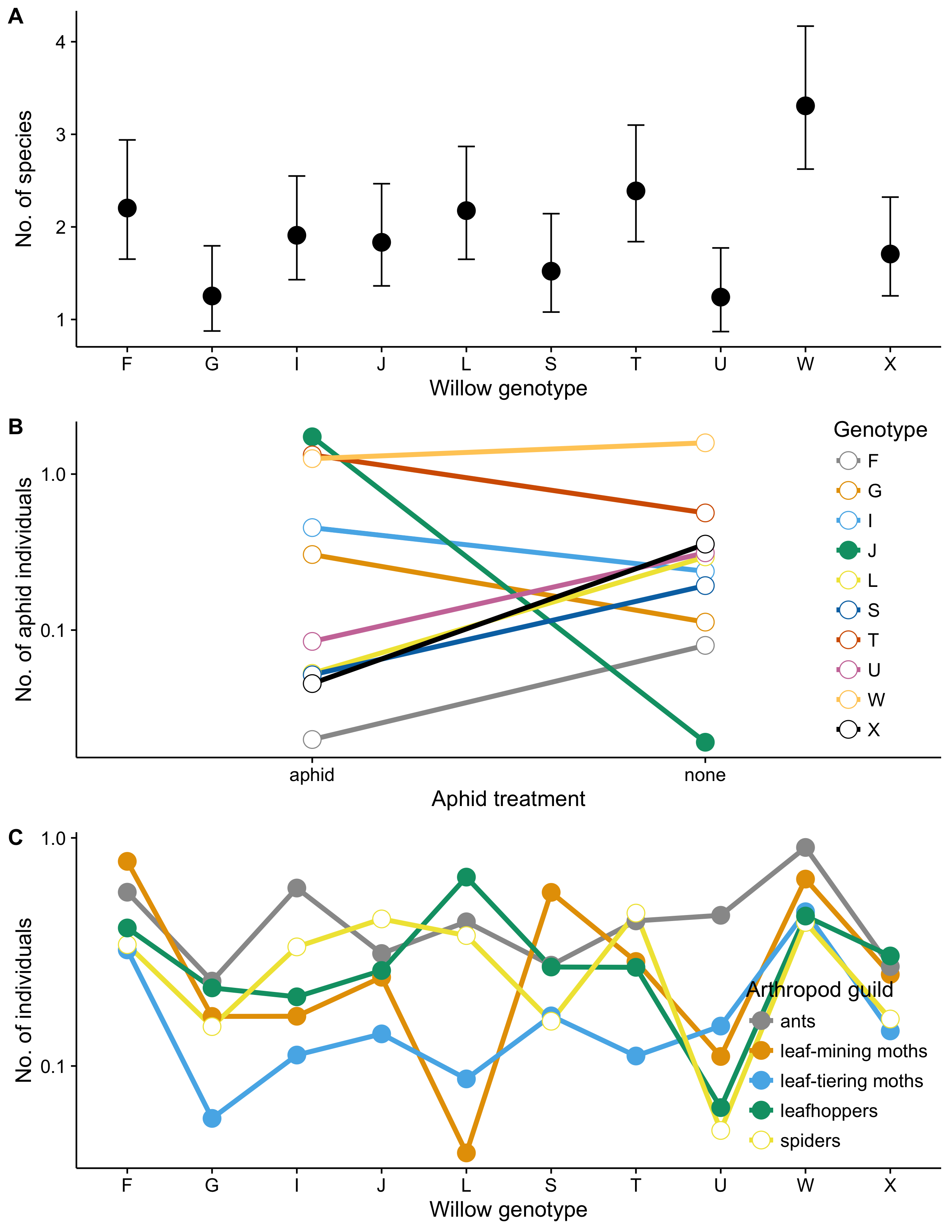
**Figures**



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**Figure Legends**

**Tables**

**Table 1**: Summary of wind experiment analyses. We report test statistics (F or Chi-square) and bold significant values.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Response | Genotype (G) | Ewind | GEwind | Eyear | GEyear | EwindEyear | GEwindEyear |
| **Plant-growth traits** |  |  |  |  |  |  |  |
| Plant height | **9.13** | **29.10** | 0.71 | **210.09** | 0.80 | **16.69** | *1.84* |
| Shoot count | **47.42** | **9.91** | 10.70 | **5.68** | **18.26** | **12.53** | 5.76 |
| Shoot length | **4.97** | **10.44** | 0.84 | **75.37** | 1.61 | 0.05 | 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 | 0.93 | 0.01 | 0.32 | - | - | - | - |
| **Arthropods** |  |  |  |  |  |  |  |
| Total abundance | **25.25** | **5.48** | 7.33 | **6.72** | 8.22 | 1.65 | 11.85 |
| Total richness | **23.63** | **13.16** | 3.43 | **6.77** | 8.72 | 0.20 | 7.84 |
| Total PIE | *15.79* | **18.81** | 6.66 | 0.34 | - | 0.16 | - |
| Hellinger distance2012 | 0.95 | 1.26 | 0.91 | - | - | - | - |
| Hellinger distance2013 | 1.14 | **5.70** | 0.69 | - | - | - | - |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Mychorrizae2013** |  |  |  |  |  |  |  |
| Total abundance | 0.80 | 0.40 | 1.03 | - | - | - | - |
| Total richness | 1.28 | 1.01 | 1.23 | - | - | - | - |
| Total PIE | 0.87 | 0.88 | 0.93 |  |  |  |  |
| Hellinger distance | **1.00** | 1.15 | 0.87 | - | - | - | - |
| **Bacteria2013** |  |  |  |  |  |  |  |
| Total abundance | 1.39 | 2.00 | 0.64 | - | - | - | - |
| Total richness | 1.35 | *4.53* | 0.87 | - | - | - | - |
| Total PIE | 1.48 | **6.03** | 1.35 |  |  |  |  |
| Hellinger distance | 0.93 | *1.38* | 0.87 | - | - | - | - |

**Table 2**: Summary of ant-aphid experiment analyses. We report test statistics (F or Chi-square) and bold significant values.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Response** | **Genotype (G)** | **Eaphid** | **Eant** | **GEaphid** | **GEant** | **EaphidEant** | **GEaphidEant** |
| **Plant-growth traits** |  |  |  |  |  |  |  |
| Plant height | **15.83** | 0.63 | 0.31 | 0.93 | 0.98 | 0.07 | 1.62 |
| Shoot count | **65.84** | *2.76* | 0.21 | 12.11 | 8.80 | **4.20** | 9.21 |
| Shoot length | **7.27** | 2.39 | 0.10 | 1.04 | 0.70 | 1.24 | 0.56 |
| **Leaf traits** |  |  |  |  |  |  |  |
| Trichome density | **38.17** | 0.44 | 0.81 | - | 9.07 | 0.43 | - |
| Water content | 1.43 | 0.01 | 0.76 | - | 0.87 | 0.44 | - |
| **Arthropods** |  |  |  |  |  |  |  |
| Total abundance | **37.34** | *3.45* | 0.98 | *16.22* | 9.63 | **7.07** | 9.82 |
| Total richness | **43.36** | 2.34 | 0.51 | 7.24 | 8.38 | 0.42 | 6.69 |
| Total PIE | 0.69 | *3.15* | 0.04 | *1.78* | 1.09 | 1.16 | 0.57 |
| Hellinger distance | **1.62** | **2.90** | 1.05 | **1.42** | 1.01 | 0.91 | 0.88 |
| Leaf-mining moth | **26.78** | 0.32 | 2.35 | 13.56 | **20.31** | **4.32** | - |
| Aphids (non-*A. farinosa*) | **24.43** | 0.01 | 0.04 | **23.16** | 6.99 | *3.63* | 8.16 |
| Leafhopper | **21.92** | 0.84 | 0.01 | 7.29 | 11.54 | 1.67 | - |
| Spiders | *16.24* | 0.01 | 0.10 | *15.39* | 11.34 | 0.01 | - |
| Ants (non-*F. obscuripes*) | **22.43** | **17.70** | 1.52 | 5.21 | 7.07 | 0.73 | - |
| Leaf-tiering moth | **23.79** | 0.81 | **9.79** | - | - | *3.77* |  |