

Genotypic variation in a foundation tree alters ecological network structure of an associated community

Matthew K. Lau^{a,b,1}, Louis J. Lamit^b, Rikke R. Naesbourg^c, Stuart R. Borrett^d, Matthew A. Bowker^e, and Thomas G. Whitham^a

^aDepartment of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ 86011, USA; ^bHarvard Forest, Harvard University, 324 N Main St, Petersham, MA 01366, USA; ^cDepartment of Biology, Syracuse University, 107 College Place Syracuse, NY 13244, USA; ^eUniversity of California Berkeley, Berkeley, CA, USA; ^fDepartment of Biology and Marine Biology, University of North Carolina Wilmington, 601 South College Road, Wilmington, NC, 28403, USA; ^gSchool of Forestry, Northern Arizona University, Flagstaff, AZ 86011, USA

This manuscript was compiled on May 20, 2020

1 **Biological evolution occurs in the context of complex networks of**
2 **interacting species in which natural selection defines the structure**
3 **of ecological networks. Fundamental to this evolutionary process**
4 **is the discovery of a genetic basis to ecological network structure.**
5 **Although previous work has demonstrated that tree genotype con-**
6 **tributes to interaction network structure at the scale of forest stands,**
7 **the contribution of tree genetics to localized interaction networks**
8 **at the scale of individual trees has not yet been explored. To test**
9 **the degree to which tree genetics can contribute to network struc-**
10 **ture we conducted quantitative modeling of interaction networks. We**
11 **constructed networks of epiphytic lichen associated with individual**
12 **trees that were a part of a long-term experimental common garden**
13 **of genotypes of (*Populus angustifolia*), a foundation species. We**
14 **found three main results. First, tree genotype significantly predicted**
15 **lichen network similarity, i.e. trees of the same genotype had more**
16 **similar lichen networks. Second, positive interactions of one lichen**
17 **species, *Caloplaca holocarpa* drove the genetically based variation**
18 **in network structure. Third, bark roughness was both predicted by**
19 **tree genotype and correlated with lichen network similarity. We con-**
20 **clude that tree genotype can influence not only the relative abun-**
21 **dances of organisms but also the interaction network structure of**
22 **associated organisms. Given that variation in network structure can**
23 **have consequences for the dynamics of communities through alter-**
24 **ing the stability of the system and modulating or amplifying perturba-**
25 **tions, these results have important implications for the evolutionary**
26 **dynamics of ecosystems.**

networks | heritability | community | genetics | lichen | cottonwood |
Populus | common garden

1 **E**volution occurs in the context of complex ecolog-
2 **ical networks. Initially, evolution in a community**
3 **context was focused on examples of highly co-evolved**
4 **pairs of species (e.g. Darwin's famous prediction of**
5 **the Sphinx Moth and Christmas Orchid) (?). How-**
6 **ever, studies of diffuse co-evolution (*sensu* (?)) (?**
7 **? ?), geographic mosaics of co-evolution (1) and**
8 **community genetics (2) have provided an in-road for**
9 **ecological network approaches (3? ?) to illuminate**
10 **a more complex perspective of the interface between**
11 **ecological and evolutionary dynamics. There is now**
12 **evidence to support that selection tends to occur**
13 **among groups of species (? ? ?) favoring the de-**
14 **velopment of small webs (? ? ?) and that genetic**

15 variation and phylogenetic relatedness contributes
16 to variation in community assembly (4) and species
17 interactions (2, 5, 6), which shapes the ecological
18 interaction networks (7).

19 Community genetics studies (8) have shown that
20 genetic variation in foundation species (9) plays a
21 significant role in defining distinct communities of in-

Significance Statement

Evolution occurs in the context of ecosystems comprised of complex ecological networks. Research at the interface of ecology and evolution has primarily focused on pairwise interactions among species and have rarely included a genetic component to analyses. Here, we use a long-term common garden experiment to reveal the effect that genotypic variation can have on networks of lichens that occur on the bark of a foundation tree species. We found that lichen interaction network structure is genetically based and primarily driven by a tree trait, bark roughness. These findings demonstrate the importance of genetic variation and evolutionary dynamics in shaping ecological networks as evolved traits. In particular, this study points to the importance of assessing the effect of foundation species genetics on the structure of interactions, given that interaction network structure has systems-level properties that could affect the response of these communities to selection.

M.L. and L.L. conceived the study, M.L. and L.L. conducted the field work, R.N. assisted in lichen identifications, M.L. wrote the first draft of the manuscript, S.B. and T.W. contributed substantively to the conceptual development, T.W. established the common garden. All authors contributed to revisions of the manuscript.

The authors have no conflicts of interest.

¹Dr. Matthew K. Lau. E-mail: matthewklau@fas.harvard.edu

teracting organisms: such as, endophytes, pathogens, lichens, arthropods, and soil microbes. Multiple studies have now demonstrated that genetic variation influences numerous functional traits (e.g., phytochemical, phenological, morphological) produces a multivariate phenotype (10) that contributes to variation in associated communities (5). The importance of genetic variation in structuring ecological systems was recently reviewed (11), and not only were many instances of strong genetic effects found in many ecosystems but the effect of intraspecific variation was at times greater than *inter*-specific variation.

Additional work has provided support for the hypothesis that not only does composition vary among genetically distinct genotypes of foundation species but that it also impacts the structure of species interactions. However, studies in the network ecology literature generally do not include a genetic component (?). And, community genetics studies have primarily focused on community composition in terms of the abundance of species (11). Multiple studies from different plant-associated communities (including *Populus*, *Solidago*, *Oenothera*, *Salix*) have examined the effect of genetic variation on trophic interactions (12–15) and generally found that increasing genotypic diversity leads to increased trophic complexity. Similarly, two other studies have examined the effect of genotypic variation on the structure of interactions between tree individuals and the associated community (3, 16) and both found that genotypic diversity generates increased network modularity (i.e. compartmentalization).

Here, we investigate how genetic variation in a foundation tree species determines the structure of a network of interactions among a community of tree associated lichen species. Previous studies have examined aspects of networks (17). Here we examine the genetic basis of network structure on a community of sessile lignicolous (i.e. bark) lichen on cottonwood trees. Using a long-term (20+ years), common garden experiment with replicated individuals of known genetic identity and a naturally established stand of *Populus angustifolia*. We focused on a model community of 9 epiphytic lichen species, as previous research has demonstrated significant compositional responses of epiphytes to genotypic variation (18, 19). In addition, the life-history characteristics of lichens, having highly localized, direct contact interactions and slow population turnover

rates, allowed us to assess interactions among lichen species on individual trees. We hypothesize that in natural systems evolution occurs in a community context involving interactions of complex networks of interacting species (1, 3, 16, 20). If correct, we expect to find that network structure is genetically based in which different plant genotypes support different interaction networks and that these interactions networks can function as indicators of ecological dynamics important for conserving biodiversity. Applying a probability-theory based network modeling approach, we constructed a set of interaction network models for the lichens associated with individual trees. Using these models, we then examined the genetic basis of the structure of these ecological networks. Based on previous community genetics studies, particularly (?) which proposed the community similarity rule, we hypothesize that trees will vary in some phenotypic traits and those trees of the same genotype will tend to have similar traits leading to similarities in lichen network structure.

Materials and Methods

Study System. The study was conducted along the Weber River, UT (USA), which is a cottonwood (*Populus* spp.) dominated riparian ecosystem. Although two native species, *Populus angustifolia* (James) and *Populus fremontii* (S. Watson), occur here and are known to hybridize, only pure or advanced generation backcrosses of *P. angustifolia* were sampled. Bark lichens have been extensively studied in this system and provide an ideal system in which to observe and model lichen interaction networks, as their sessile nature permits accurate identification of individuals (21).

A long-term, common garden experiment was used to isolate the effect of tree genotype from the effect of the localized microenvironment associated with each individual and spatial autocorrelation. Established in 1992, asexually propagated clones of genotyped *P. angustifolia* individuals were obtained from wild collections and planted in fully randomized design at the Ogden Nature Center, Ogden, UT. From the population of established individuals in the common garden, we chose a total of thirteen genotypes, replicated between 3 and 8 times each, for sampling.

Bark Lichen and Trait Observations. On each tree, presence or absence of each lichen species was assessed in 50 total 1 cm² cells arrayed in a checkerboard pattern. Given the small size and sessile nature of lichens, we were able to rapidly assess lichen interactions by quantifying thalli

in close contact. Sampling was restricted to the northern aspect of the trunk to maximize the abundance of lichen and control for the effect of trunk aspect. Two adjacent 10 cm² quadrats centered at 50 cm and 85 cm from ground level were sampled (Fig 1 A and B). The observed lichen community included (abbreviations are given for species present in study): Xg = *Xanthomendoza galericulata*, Xm = *X. montana*, Ch = *Caloplaca holocarpa*, Cs = *Candelariella subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*, Pm = *Phyciella melanchra*, Pa = *Physcia adscendens*, Pu = *Physcia undulata*. Several other species were not observed in the present study but are known to occur in this region: *Phaeophyscia orbicularis*, *Phaeophyscia ciliata*, *Melanelia subolivacea*, *Meanelia elegantula*.

The cell size and checkerboard sampling pattern was chosen to isolate the individuals in each cell. In a previous survey of lichen thallus size in this common garden, we had observed a median thallus size of 0.12 ± 0.001 cm² (S.E.) (see Supporting Information). Based on the median thallus size, we expected thalli observed in each cell to generally be spatially independent of thalli present in other cells but exposed to similar micro-environmental conditions created by the bark and the location of the sampling area on an individual tree. Therefore, we were confident in treating the cell-wise observations in quadrats as independent with respect to lichen-lichen interactions.

We also measured several bark traits for each tree including, bark roughness, condensed tannin, carbon and nitrogen concentrations and pH. **ADD METHODS FROM JAMIE.**

Lichen Network Modeling and Analysis. For each tree, repeated observations of lichen were made in order to construct replicated interaction networks for each genotype. We quantified the presence of lichen in the 1 cm² cells of individual trees of *P. angustifolia*. Unipartite networks were generated using the conditional probabilities of each species pair, i.e. the probability of observing one species given an observation of another species $P(S_i|S_j)$, based on the method developed by (22). To calculate conditional probabilities, we quantified the individual probabilities of species occurrences $P(S_i)$ and the joint probability of co-occurrences $P(S_i, S_j)$ using the frequencies of each species and their co-occurrences. We were then able to calculate the conditional probabilities of each species pair as $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$, based on the axioms of probability. This yielded a matrix that could possibly be asymmetric, i.e. $P(S_i|S_j)$ does not have to be equal to $P(S_j|S_i)$. Another important property of this matrix is that the diagonal (S_{ii}) was equal to one for all species present and zero for species that were not observed in any cell.

We then applied an analytical procedure to remove non-significant links between species. This procedure determines if the joint probability of a species pair (i.e. $P(S_i, S_j)$) is different from zero (Fig. 2). Here, a confidence interval $CI_{95\%}$ is calculated as

$E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$, where the expected frequency of co-occurrences $E(S_i S_j)$ is the total number of cells surveyed (N) times the independent probabilities of each species $P(S_i) * P(S_j)$, $Z_{95\%}$ is the Z-score for 95% from a Z-distribution and the expected variance of $E(S_i S_j)$ is the total number of cells times the expected probability of $S_i S_j$ and its compliment (i.e. $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$). If the observed number of co-occurrence falls outside of the confidence interval, the joint probability $P(S_i, S_j)$ is determined to be equal to the product of the individual probabilities (i.e. $P(S_i)P(S_j)$), and the conditional probability reduces to the individual probability of that species $P(S_i)$. Therefore, unless the co-occurrence of a species pair falls outside the confidence interval, the probability that the observation of one species given the other is no different than simply observing that species alone. This enables us to remove links from a given network by re-scaling the resulting conditional probabilities by subtracting the individual probabilities from the conditional probabilities (i.e. how different the conditional probability is from the independent probability), which makes any species with a non-significant conditional probability zero. The resulting matrix ($\mathbf{D} = D_{ij}$) can be interpreted as how one species impacts another with zero being no effect and values less than or greater than zero interpreted as negative and positive effects, respectively. Here, we will refer to this matrix (\mathbf{D}) as an interaction matrix with the properties that it can be asymmetric (i.e. P_{ij} does not necessarily equal P_{ji}), and the diagonal (P_{ii}) is zero (i.e. a species does not influence its own probability of being observed).

Network Metrics. To quantify the structural variation of lichen networks we calculated several metrics at both the node and whole-network level. For individual nodes (i.e. species) in each network, we calculated both the degree Eq. (1) and the centrality. We also calculated two similar global network metrics: degree and centralization. The first was network degree, which is a count of the total number of links in a network. As the networks contained not only positive and negative connections, as well as directional connections (both in-coming and out-going), we calculated the same network metrics for all combinations of these types of connections in each network. Although there are many more possible network metrics that could have been examined, we chose to focus on a restricted set for the sake of clarity. Also, degree and centrality form the basis of many other network metrics.

ADD EQUATIONS FOR METRICS

- | | |
|---|---|
| <p>170</p> <ul style="list-style-type: none"> • Node degree • Node centrality • Network degree | <p>[1] 225</p> <p>226</p> <p>227</p> <p>228</p> |
|---|---|



Fig. 1. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees at two heights, 40cm and 80cm from the ground (A and B, respectively). (C) a photo of a typical community of bark lichen species interacting on the trunk of a cottonwood tree, including one of the more abundant species, *Xanthomendoza galericulata*, in the center. (D-K) shows the other lichen species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp., *Caloplaca holocarpa*, *Physcia adscendens*, *Phyciella melanchra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesbourg (D-K).

- Centralization
- In vs out
- Pos vs neg

To calculate separate metrics for positive and negative links, we applied methods for calculating the centrality accounting for the sign differences (?? and Borgatti 2014). We used the `signnet` package version ????, which is available at ???.

Statistical Analyses, Software and Data. We used a combination of parametric and non-parametric, permutation based frequentist statistical analyses to test for the effects of genetic variation on lichen communities and their interaction networks. To assess the effect of genotype on univariate responses, we used additive, random effects models with Restricted Maximum Likelihood (REML). We used a combination of Least Squares Regression, Analysis of Variance (ANOVA) and correlation tests to quantify and test for the relationship among other variables. Bark roughness, lichen cover and species richness were square-root transformed to meet the assumptions of homogeneity of variance and normality for these tests.

For multivariate response variables, such as lichen community composition and network structure, we used distance based multivariate statistical approaches, including Permutational Analysis of Variance (PERMANOVA) and Mantel tests. To quantify the similarity of lichen networks among individual trees, we calculated the pairwise Euclidean distance of the **D** interaction matrices among 231 pairs of trees.

For visualization of multivariate patterns, we used Non-metric Multi-Dimensional Scaling (NMDS) (?) to produce dimensionally reduced ordinations of these multivariate responses and fitted vectors for continuous predictor variables to the ordinated values (?). Using random initial configurations with a maximum of 500 iterations and a change in stress threshold of less than 10^{-12} . Final configurations has the lowest stress with at most a stress level of 0.10.

For each network, we also calculated metrics that measure different structural aspects. Although there are many other metrics, for the sake of simplicity we focus on a subset that represent several interesting features of network structure (see (23)). We calculated the number of interactions or “links” in each network, which provides a

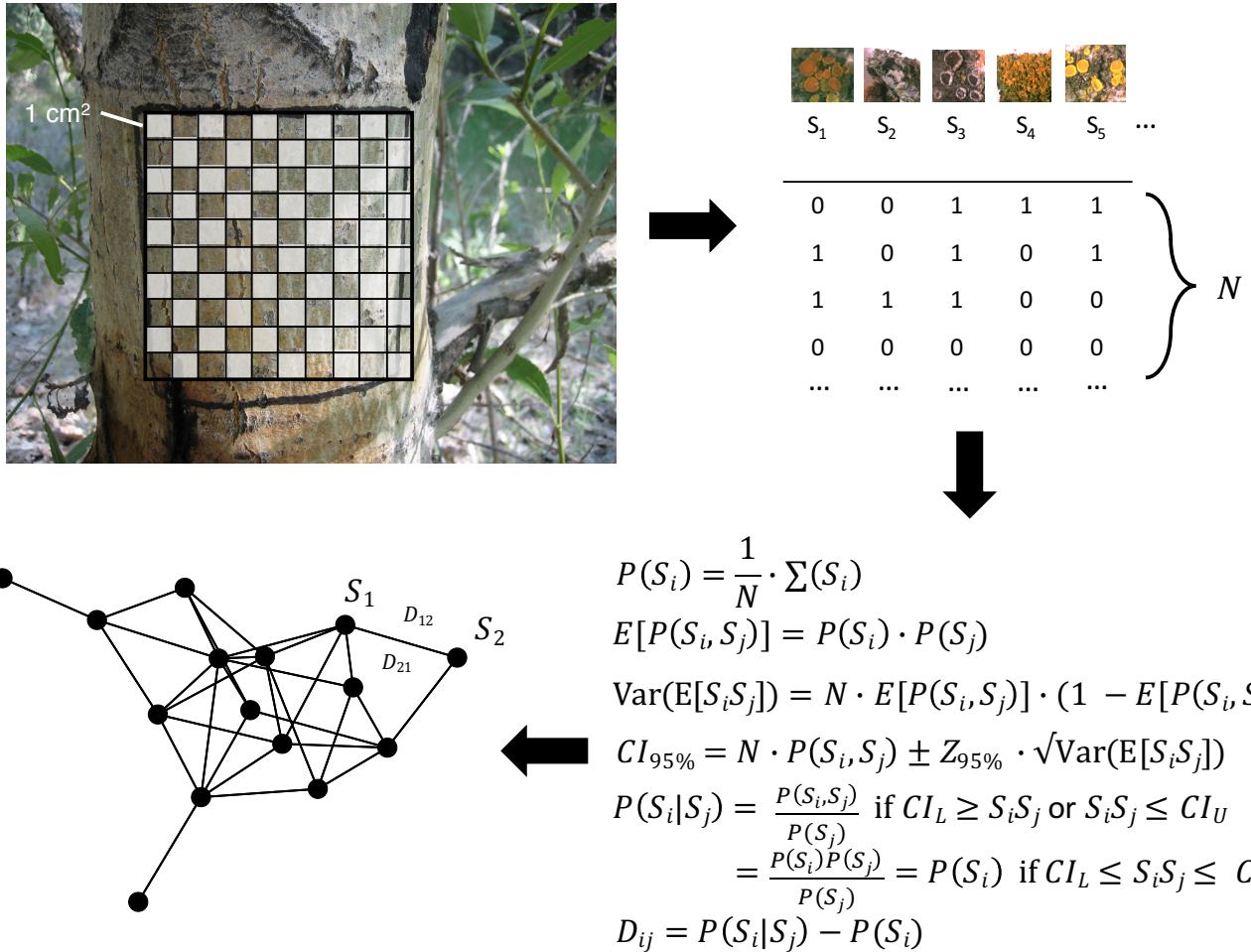


Fig. 2. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (22), we calculated the conditional probabilities, $P(S_i | S_j)$, for all species pairs and removed (i.e. set equal to zero) species pairs whose joint probabilities, $P(S_i, S_j)$, were not significant using a confidence interval based comparison of their observed co-occurrence frequency, $S_i S_j$, to that expected due to chance alone, $E[P(S_i, S_j)] = P(S_i) P(S_j)$, and $P(S_i | S_j)$ reduces to $P(S_i)$, the observed individual probability of species S_i . In the context of these networks, asymmetry and positive/negative valued connections are distinct quantities. In-coming and out-going connections can be interpreted as “influenced by” and “influenced”, respectively; while positive and negative should be seen as one species increasing or decreasing, respectively, the probability of another species’ occurrence.

measure of the size of the network (24?). We also calculated the centralization of each network, which measures the evenness of the distribution of interactions among the species in the network (?). In a network with a low level of centralization species have similar amount of interaction in the network, while a network with a high level of centralization tends to have one or small number of species that interact with other species. We used a related function to calculate the centrality of each species (i.e. node level centrality) in each network as well. The modularity of each network was also quantified using a weighted algorithm (?), which measures the degree to which a given network is divided into groups of species more connected to each other than other species. As with the other response variables, the number of links was log-transformed and both modularity and centralization scores were fourth-root transformed to meet variance and

normality assumptions.

All code and data for the project are openly available online. Code and data are available at github.com/ecgen/comgen. The project is also archived via Zenodo at zenodo.com/doi/XXXXXX. All analyses were conducted using the programming language R version 3.6.1 (R Development Core Team 2019).

Results

Tree genotype influenced lichen network structure. Tree genotype significantly predicted the structural similarity of lichen networks (PERMANOVA: $\text{Pseudo-}F_{9,27} = 3.58$, $H^2 = 0.41$, $p\text{-value} = 0.0537$) (Fig. 4). Overall network level metrics responded significantly to tree genotype (Table 1), including net-

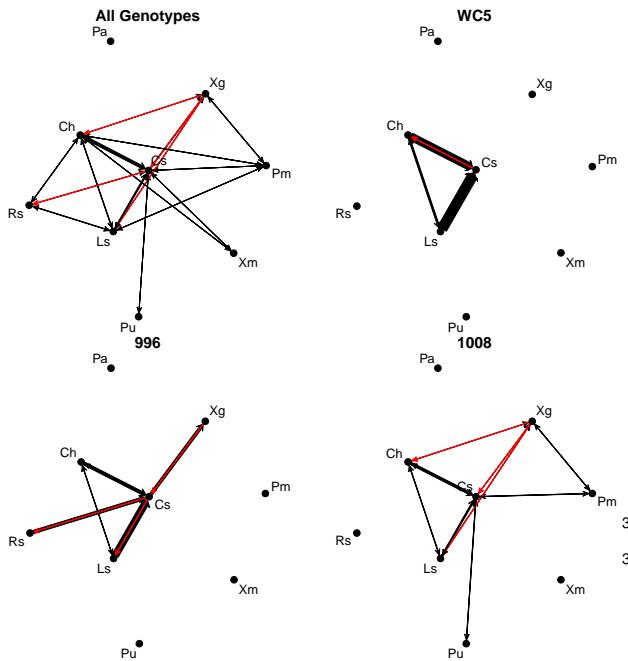


Fig. 3. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive) of interactions are shown as edges between species (abbreviated by the first letter of the genus and specific epithet), which are scaled by their magnitude. The sign of the interaction is indicative of greater (positive) or lesser (negative) paired occurrences than expected relative to the overall frequency of occurrence of each species. Ecologically, the links in the network are likely the product of multiple types of interactions (e.g. mutualism, parasitism, competition, facilitation) that could vary over both space and time.

	response	statistic	H2	p-value
Lichen Network Similarity	3.5821	0.4130	0.0537	
Degree	3.5175	0.3156	0.0255	
Degree (positive)	3.6925	0.3242	0.0229	
Degree (negative)	0.0327	0.0318	0.3859	
Centralization	4.0444	0.3305	0.0184	
Centralization In-Degree	4.4812	0.3487	0.0142	
Centralization In-Degree (positive)	3.9852	0.3309	0.0190	
Centralization In-Degree (negative)	0.3304	0.1057	0.2508	
Centralization Out-Degree	3.8615	0.3193	0.0205	
Centralization Out-Degree (positive)	3.5585	0.3119	0.0248	
Centralization Out-Degree (negative)	0.0862	0.0513	0.3446	

Table 1. Genotypic effects on the associated lichen network structure.

centrality was the main species to exhibit a significant response to tree genotype in terms of positive centrality for both the in-coming ($RLRT = 3.61, H^2 = 0.32, p\text{-value} = 0.0240$) and out-going ($RLRT = 3.13, H^2 = 0.30, p\text{-value} = 0.0327$) perspectives, but not for either negative centrality metrics in-coming ($RLRT = 0, H^2 = 0, p\text{-value} = 1$) or out-going ($RLRT = 0, H^2 = 0, p\text{-value} = 0.4543$). None of the other species' centralities showed a genotypic response (Supplementary Table 6) with the exception of *X. montana* ($RLRT = 2.92, H^2 = 0.32, p\text{-value} = 0.0375$); however, the centrality of *X. montana* was much lower overall relative to *C. holocarpa* and the variation in *X. montana* centrality was restricted to two genotypes (Fig. 5).

Genotype indirectly influenced lichen network centralization via the genetically based variation in bark roughness. The percent of rough bark was the only tree trait that displayed a significant response to genotype ($RLRT = 4.8526, H^2 = 0.3221, p\text{-value} = 0.0113$). None of the other bark traits, condensed tannins ($RLRT = 0.0007, H^2 = 0.0041, p\text{-value} = 0.4439$), pH ($RLRT = 0.00, H^2 = 0.00, p\text{-value} = 1.0000$) or carbon-nitrogen Ratio ($RLRT = 0.0000, H^2 = 0.0000, p\text{-value} = 1.0000$), showed a significant response to tree genotype and none other than bark roughness were correlated with network similarity (Supplementary Table 5); therefore, we focused our analysis on bark roughness. We found that bark roughness was significantly correlated with network similarity (PERMANOVA: Pseudo- $F_{1,32} = 13.029, R^2 = 0.26, p\text{-value} = 0.0096$) and other lichen network metrics, including negative correlations with overall network degree ($df = 35, t = -2.13, r = -0.34, p\text{-value} = 0.04$) and centralization ($df = 35, t = -2.52, r = -0.39, p\text{-value} = 0.02$). To determine how

work degree ($RLRT = 3.52, H^2 = 0.32, p\text{-value} = 0.0255$) and centralization including both in-coming and out-going links ($RLRT = 4.04, H^2 = 0.33, p\text{-value} = 0.0184$) or when separated into in-coming only ($RLRT = 3.9852, H^2 = 0.3309, p\text{-value} = 0.0190$) or out-going only ($RLRT = 3.8615, H^2 = 0.3193, p\text{-value} = 0.0205$). Metrics including only positive links also showed a significant effect of tree genotype, including positive degree ($RLRT = 3.6925, H^2 = 0.3242, p\text{-value} = 0.0229$), positive in-going centralization ($RLRT = 4.4812, H^2 = 0.3487, p\text{-value} = 0.0142$). Metrics calculated with negative links were not significant, including degree (negative) ($RLRT = 0.0327, H^2 = 0.0318, p\text{-value} = 0.3859$) and both in-coming (negative) ($RLRT = 0.3304, H^2 = 0.1057, p\text{-value} = 0.2508$) and out-going centralization (negative) ($RLRT = 0.0862, H^2 = 0.0513, p\text{-value} = 0.3446$).

The genetic response of network centralization was driven by variation in *Caloplaca holocarpa*. Centrality varied significantly among species ($F_{8,324} = 7.99, R^2 = 0.16, p\text{-value} << 0.0001$). *Caloplaca holocarpa*

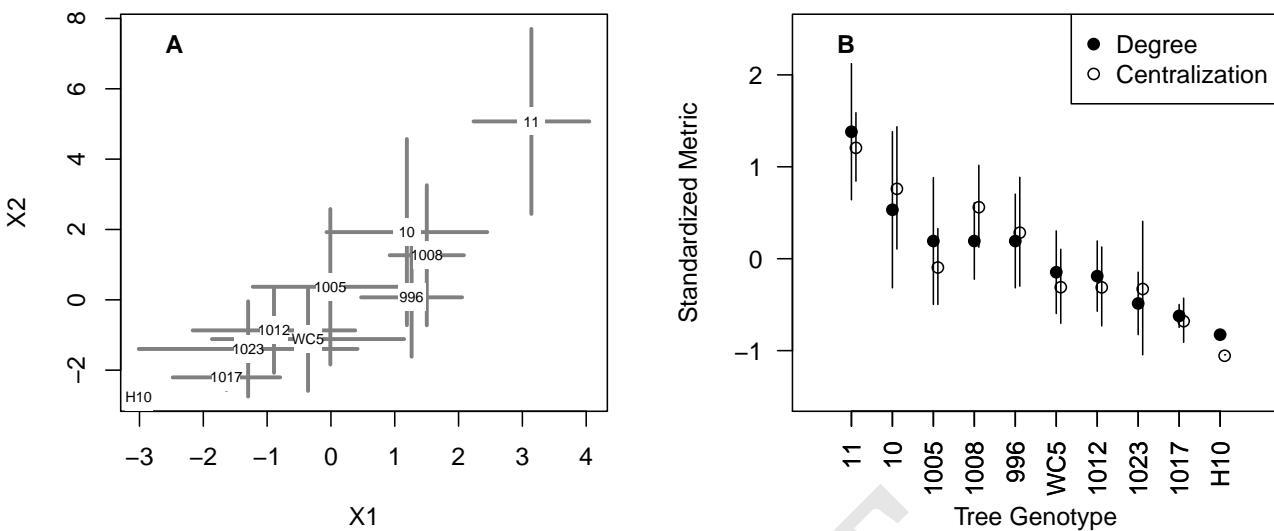


Fig. 4. The similarity of lichen networks varied among tree genotypes. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks (± 1 S.E.). Genotype centroids that are closer together tend to have more similar lichen network structure. B. Plot showing the standardized ($\frac{x - \bar{x}}{\sigma}$) means (± 1 S.E.) for the two of the genetically based lichen network metrics: overall degree (i.e. total number of links) and centralization, which is a measure of the dominance of one species in the network.

much of the effect of bark roughness was genetically based, we used the residual values from regressions of network degree and centralization in tests of the effect of tree genotype and found no significant effect of tree genotype for either degree ($RLRT = 0.00$, $H^2 = 0.00$, *p-value* = 1.0000) or centralization ($RLRT = 0.00$, $H^2 = 0.00$, *p-value* = 1.0000), suggesting that the observed relationship between bark roughness and lichen network structure was largely genetically based (Fig. 6).

Discussion

We found that tree genotype influenced lichen network structure in the experimental cottonwood forest. Network similarity and metrics of network structure tended to be more similar on trees of the same genotype. Generally, this genetic effect was manifested in positive interactions and largely driven by *C. holocarpa*. Bark roughness was the primary genetically based trait driving network structure. The genetically based trait, bark roughness, was the main driver of network variation. Not only was bark roughness the only trait observed to be genetically based, it was correlated with network structure and residual variation from this correlation was not explained by tree genotype. These results have important implica-

tions for the potential influence of genetically based variation in ecosystems with networks of interacting species.

Differences in distributions below the quadrat scale are leading to shifting patterns of interactions among lichen species, largely increased positive incoming and out-going interactions. It could also be that some other variable correlated with bark roughness is altering the quality (i.e. how) the lichen species are interacting, that is as opposed to simply the "quantity" of interactions. Bark roughness effect was negative, possibly serving the role that other lichen play in facilitating the success of new propagule attachment and the growth of establishing thalli. This is supported by the patterns overall being positive, including *C. holocarpa*'s centrality being positive both in and out. We don't know specific micro-scopic dynamics, such as photobionts, mycobionts, endolichenic fungi and bacteria, but variation in these underlying interactions could also be playing a role. Also, bark roughness had previously been shown to be an important tree trait influencing bark lichens (21) that is under strong genetic control (25).

There are important functional ramifications of genetically based variation in network structure. First, even if the composition of the communities is the

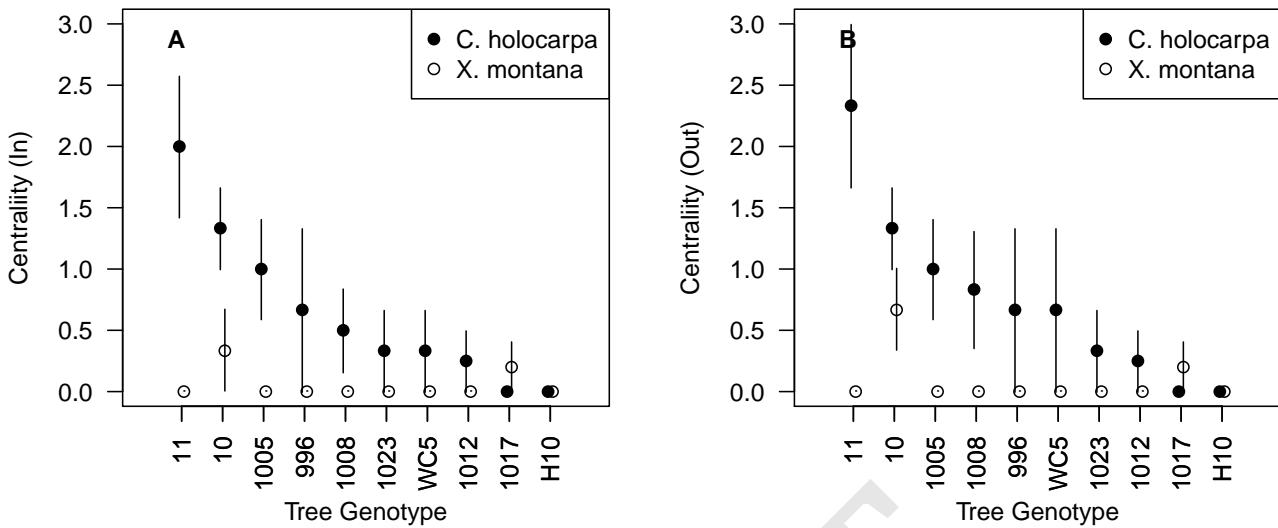


Fig. 5. Dot-plots showing the mean (dot) and ± 1 SE of in-degree (A) and out-degree (B) centrality for two species, *C. holocarpa* and *X. montana*. *Caloplaca holocarpa* centrality was highly variable among genotypes. *Xanthomendoza montana* centrality, both in- and out-degree, was only non-zero for two genotypes, and only out-degree centrality displayed a significant response to genotype.

same among individuals and genotypes, interactions may not be. We didn't observe compositional differences using the same data from which the lichen networks were derived. If we only had our composition dataset from this study, we would have concluded no response of the lichen community to tree genotype, even though the underlying interactions among lichen species does vary among genotypes. Community composition of lichen has previously been observed to be different among tree genotype in the same experimental garden, though this was observed with a larger sampling of total area and quadrats per tree. Regardless, this could result in a situation in which abundance based investigations of community-level genetic effects may miss important variation in the interactions among individuals in these communities, leading to an underestimate of genetic effects in ecosystems. It is possible that these underlying differences in interactions among lichen could lead to differences in community composition at a future point in time, however, this is not needed for evolutionary dynamics to play out.

Second, following on the previous point, genetic diversity could be influencing the stability of communities through the effects on the structure of interactions. Some network structures are likely to be more stable, either in response to disturbance or via

self-organized dynamics. For example, centralized networks, although more efficient, are theorized to be more susceptible to targeted attacks on the center of the network. For example, consider a forest with two genotypes that support lichen communities that are similar in total abundances of each species but differ in terms of the structure. Extensions of game theory to evolutionary biology have demonstrated that network structure can lead to variation in evolutionary dynamics. Some structures tend toward dominance and dampening of selection, while others lead to amplification of selection (Newman). One class of networks that are theorized to have amplifying effects on networks have "star" shapes with one or a few species at the center and radiating interactions out from the central core (Leiberman). This is structurally what we have observed with the networks that tend to occur on some of the genotypes in our study, i.e. the more centralized networks. It is possible that these more centralized networks could function as hot-spots of evolutionary dynamics resulting from the amplifying effect the network structure fostered on that tree genotype.

Altering the structure of interaction networks presents a means for genetic effects to be magnified within the system of interacting species. For example, (3) showed that the genetics based interac-

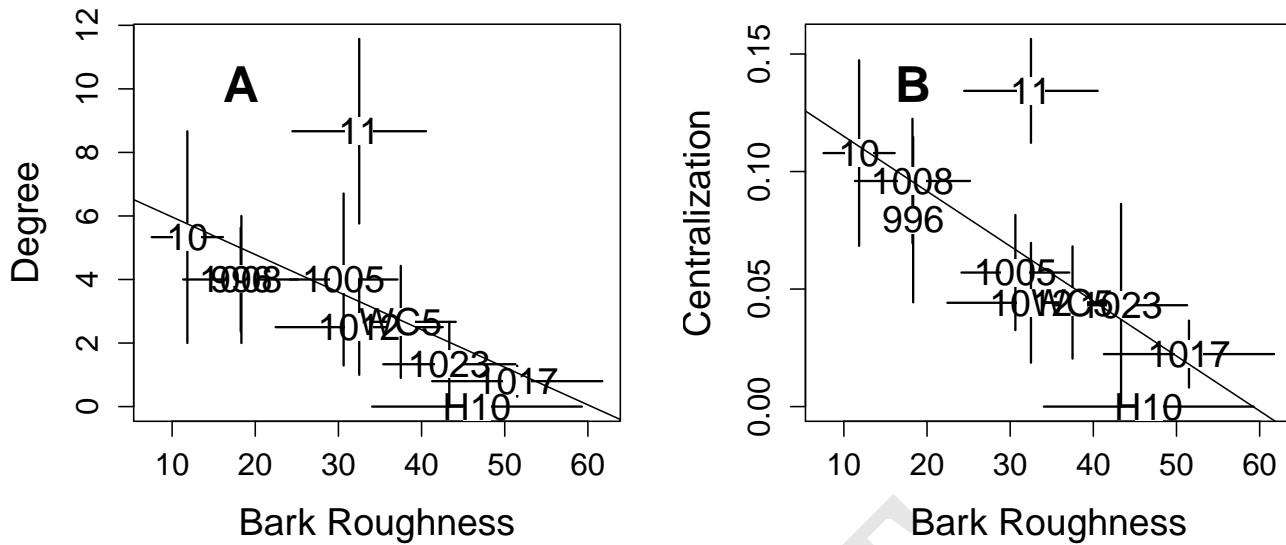


Fig. 6. Bivariate plots of the relationship between bark roughness and three network metrics: A) degree and B) centralization. Each plot displays the genotype mean \pm 1 S.E. for both variables and a least-squares regression calculated using the genotype means.

tions of aphid resistant and aphid susceptible trees resulted in different interaction networks of their associated arthropod communities composed of 139 species. At the scale of ecosystems, trophic networks or food webs direct and control the rates of energy and nutrient flux (26). Furthermore, in a predator-prey-plant study, Smith (13), showed that the interactions among species across trophic levels depended on plant genotype. Also, work by (27–29) observed consistent patterns of centralized interactions of species modules focused around hubs of plant-fungal interactions. In other words, a small number of plant and fungal symbionts tended to have disproportionate numbers of interactions with other species and likely are the drivers in determining community assembly, structure and dynamics.

There are several important points to consider with regard to the generalization of the observed genetically based response of the lichen networks. Body size and sessile nature of lichen important to observing genotype responses. As bark lichen individuals do not move, but grow in a largely two dimensional plane, these communities and their interactions occur in the highly localized context of the tree's bark surface. Lichen individuals are many orders of magnitude smaller than the tree individual and the life-span of a tree is many times that of a

lichen. For these reasons, any genetic effects on these communities is not dampened by the movement of individuals and the mixing of the effect of different tree genotypes on the lichen community, as might occur for more mobile species (e.g. insects and birds). We only looked at lichen, other species whose distribution, abundance or interactions respond to tree genotype, such as epiphytic plants (e.g. moss and liverworts), algae or insects, could be playing a role. Other traits could also be playing a role, such as traits that are correlated with bark roughness, such as micro-aspect, albedo, moisture, etc.

- Barbour 2018 Trait plasticity is more important than genetic variation in determining species richness of associated communities. Synthesis: These results indicate that trait plasticity can be a dominant driver of above- and below-ground biodiversity.
- Barbour 2015 Multiple plant traits shape the genetic basis of herbivore community assembly. Synthesis: Taken together, our results support that the genetic basis of herbivore community assembly occurs through a suite of plant traits for different herbivore species and feeding guilds.
- Busby 2019 Contingency rules for pathogen competition and antagonism in a genetically based,

521 plant defense hierarchy. Synthesis: Our results⁵⁶⁷
522 point to a *Populus* defense hierarchy with re-
523 sistance genes on top, followed by pathogen
524 competition, and finally pathogen antagonism
525 by endophytes. We expect these rules will help
526 to explain the variation in pathogen antagonism
527 that is currently attributed to context depen-
528 dependency.

- 529 • Barker 2019 Linking plant genes to insect com-
530 munities: Identifying the genetic bases of plant
531 traits and community composition. Synthesis:
532 These findings support the concept that partic-
533 ular plant traits are the mechanistic link between
534 plant genes and the composition of associated
535 insect communities.
- 536 • Cooper 2018 Genotypic variation in phenological
537 plasticity: Reciprocal common gardens reveal
538 adaptive responses to warmer springs but not to
539 fall frost. Synthesis: Trees transferred to warmer
540 climates generally showed small to moderate
541 shifts in an adaptive direction, a hopeful result
542 for climate change. Trees experiencing cooler
543 climates exhibited large, non-adaptive changes,
544 suggesting smaller transfer distances for assisted
545 migration.
- 546 • Whipple 2018 Epigenetic inheritance across the
547 landscape. Synthesis: Transgenerational epi-
548 genetic variation may alter the interpretation
549 of landscape genomic studies that rely upon
550 phenotypic analyses, but should have less influ-
551 ence on landscape genomic approaches that rely
552 upon outlier analyses or genome-environment
553 associations.

554 Although our study was conducted with a commu-
555 nity of lichens, these results should be generalized
556 to other groups of diverse organisms around the
557 world that also exhibit significant genetic signals at
558 the community level (30, 31). In the face of the
559 high degree of complexity and potential context de-
560 pendence of ecological processes, the current study
561 points to the utility of considering the spatial and
562 temporal scales of interactions, as discussed to some
563 in previous studies (32–34). In the present study,
564 we found that community assembly processes, such
565 as environmental filtering and species interactions,
566 are genetically based. This is likely due, in part,
567 to the large difference in the differences in size and
568 longevity of the lichen and cottonwood individuals
569 with the trees determining the environment in which
570 the lichen occur. We suggest that future work would
571 be aided by determining these modules within the
572 biotic community that include species with similar
573 differences in body-size and time-scales. As heritable
574 variation is the raw material for natural selection
575 to act upon, a genetic basis for interaction network
576 structure indicates evolutionary dynamics should be
577 considered at the community level and that con-
578 serving genetic variation is important to consider in
579 efforts to restore or preserve complex species interac-
580 tions and their associated ecosystem functions (35).
581 With such findings, it appears that we are closer to
582 understanding the evolutionary drivers of Darwin's
583 entangled bank and the interconnectedness of species
584 in complex communities.

585 Future work should consider the potential influ-
586 ence on evolutionary dynamics of the associated com-
587 munities. The network of interactions of species that
588 are strongly influenced by a foundation species, could
589 amplify the effects of genotype, this serves as a means
590 for genetic effects to increase rather than diffuse
591 through an ecosystem either through space or over
592 time, as has been proposed in the construction of the
593 genetic diffusion hypothesis. Altered abundances can
594 lead to differences in interactions Genotype effects
595 on abundances of individual abundances may cancel
596 out. Specifically for asexually reproducing species,
597 such as many lichen are, shifting interaction frequen-
598 cies could lead to evolutionary outcomes, given the
599 potential to take-up symbionts and genetic material
600 from thalli that they come into contact with. Alter-
601 ing interaction frequencies could differences in the
602 frequencies the exchange of genetic materials among
603 lichen that could then be passed on to vegetative
604 and possibly sexually produced reproductive propa-
605 gules. The larger scale (stand or region) effects of
606 these "evolutionary units" on each tree would de-
607 pend on the connectivity and rate of movement of
608 propagules among trees per the geographic mosaic
609 of co-evolution hypothesis (1, 36).

610 **ACKNOWLEDGMENTS.** This work was supported by
611 the National Science Foundation grant (DEB-0425908)
612 and Integrative Graduate Research Traineeship (IGERT)
613 fellowships for M.L. and L.L. The Ogden Nature Center
614 staff helped to maintain the common gardens. Lichen
615 sampling was supported by Todd Wojtowicz, Luke Evans
and David Solance Smith.

- 617 1. Thompson JN (2013) *Relentless Evolution*. (University of Chicago Press), p. 499.
- 618 2. Whitham TG, et al. (2006) A framework for community and ecosystem genetics: from genes
619 to ecosystems. *Nature reviews. Genetics* 7:510–523.
- 620 3. Keith AR, Bailey JK, Lau MK, Whitham TG (2017) Genetics-based interactions of foundation
621 species affect community diversity, stability and network structure. *Proceedings of the Royal
622 Society B: Biological Sciences* 284(1854):20162703.
- 623 4. Crutsinger GM (2016) A community genetics perspective: Opportunities for the coming
624 decade. *New Phytologist*.
- 625 5. Bailey JK, et al. (2009) From genes to ecosystems: a synthesis of the effects of plant genetic
626 factors across levels of organization. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 364(1523):1607–16.
- 627 6. Moya-Laraño J (2011) Genetic variation, predator-prey interactions and food web structure.
628 *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*
629 366(1569):1425–37.
- 630 7. Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J (2007) Non-random co-
631 extinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156):925–8.
- 632 8. Lamit LJ, et al. (2015) Genotype variation in bark texture drives lichen community assembly
633 across multiple environments. *Ecology* 96(4):960–971.
- 634 9. Ellison AM, et al. (2005) Loss of foundation species: consequences for the structure and
635 dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3(9):479–486.
- 636 10. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic,
637 ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype
638 in a foundation tree species. *Oecologia* 170:695–707.
- 639 11. Des Roches S, et al. (2018) The ecological importance of intraspecific variation. *Nature
640 Ecology and Evolution* 2(1):57–64.
- 641 12. Johnson MTJ (2008) Bottom-up effects of plant genotype on aphids, ants, and predators.
642 *Ecology*.
- 643 13. Smith DS, Bailey JK, Shuster SM, Whitham TG (2011) A geographic mosaic of trophic inter-
644 actions and selection: trees, aphids and birds. *Journal of evolutionary biology* 24(2):422–9.
- 645 14. Smith DS, et al. (2015) Introduced elk alter traits of a native plant and its plant-associated
646 arthropod community. *Acta Oecologica* 67:8–16.
- 647 15. Barbour MA, et al. (2016) Genetic specificity of a plant-insect food web: Implications for
648 linking genetic variation to network complexity. *Proceedings of the National Academy of
649 Sciences of the United States of America* 113(8):2128–2133.
- 650 16. Lau MK, Keith AR, Borrett SR, Shuster SM, Whitham TG (2015) Genotypic variation in founda-
651 tion species generates network structure that may drive community dynamics and evolu-
652 tion. *Ecology* 97(3):15–0600.
- 653 17. Barbour MA, et al. (2019) Trait plasticity is more important than genetic variation in determin-
654 ing species richness of associated communities. *Journal of Ecology* 107(1):350–360.
- 655 18. Winfree R, Gross BJ, Kremen C (2011) Valuing pollination services to agriculture. *Ecological
656 Economics* 71:80–88.
- 657 19. Zytnyska SE, Fay MF, Penney D, Preziosi RF (2011) Genetic variation in a tropical tree
658 species influences the associated epiphytic plant and invertebrate communities in a com-
659 plex forest ecosystem. *Philosophical transactions of the Royal Society of London. Series B,
660 Biological sciences* 366:1329–1336.
- 661 20. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric Coevolutionary Networks Facilitate
662 Biodiversity Maintenance<url>10.1126/science.1123412</url>. *Science* 312:431–433.
- 663 21. Lamit L, et al. (2011) Genetically-based trait variation within a foundation tree species influ-
664 ences a dominant bark lichen. *Fungal Ecology* 4(1):103–109.
- 665 22. Araújo MB, Rozenfeld A, Rahbek C, Marquet PA (2011) Using species co-occurrence net-
666 works to assess the impacts of climate change. *Ecography* 34:897–908.
- 667 23. Lau MK, Borrett SR, Baiser B, Gotelli NJ, Ellison AM (2017) Ecological network metrics:
668 opportunities for synthesis. *Ecosphere* 8(8):e01900.
- 669 24. Lau MK, Borrett SR, Hines DE, Singh P (2015) enaR: Tools for Ecological Network Analysis.
- 670 25. Bdeir R, et al. (2017) Quantitative trait locus mapping of Populus bark features and stem
671 diameter. *BMC Plant Biology*.
- 672 26. Borgatti SP, Everett MG (2006) A Graph-theoretic perspective on centrality. *Social Networks*
673 28:466–484.
- 674 27. Toju H, et al. (2017) Species-rich networks and eco-evolutionary synthesis at the metacommunity
675 level.
- 676 28. Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS (2016) Network modules and hubs
677 in plant-root fungal biomes. *Journal of the Royal Society Interface*.
- 678 29. Toju H, Guimaraes PR, Olesen JM, Thompson JN (2014) Assembly of complex plant-fungus
679 networks. *Nature Communications*.
- 680 30. Rowntree JK, Shuker DM, Preziosi RF (2011) Forward from the crossroads of ecology and
681 evolution. *Philosophical transactions of the Royal Society of London. Series B, Biological
682 sciences* 366(1569):1322–8.
- 683 31. Whitham TG, et al. (2012) Community specificity: Life and afterlife effects of genes.
- 684 32. Bangert RK, et al. (2006) A genetic similarity rule determines arthropod community structure.
685 *Molecular ecology* 15:1379–1391.
- 686 33. Zook AE, Eklof A, Jacob U, Allesina S (2010) Food webs: Ordering species according to
687 body size yields high degree of intervality. *Journal of theoretical biology* 271(1):106–113.
- 688 34. Zytnyska SE, Khudr MS, Harris E, Preziosi RF (2012) No Title. *Oecologia* 170(2).
- 689 35. Evans DM, Pocock MJO, Memmott J (2013) The robustness of a network of ecological net-
690 works to habitat loss. *Ecology letters* 16:844–52.
- 691 36. Barbour RC, et al. (2009) A geographic mosaic of genetic variation within a foundation tree
692 species and its community-level consequences. *Ecology* 90(7):1762–1772.

Supplementary Materials

693

Tables.

694

	df	SS	R2	F	p-value
geno	9.0000	44078.1327	0.5442	3.5821	0.0537
Residual	27.0000	36915.4605	0.4558		
Total	36.0000	80993.5932	1.0000		

Table 1. PERMANOVA Pseudo-F Table of lichen network similarity to genotype.

	response	statistic	H2	p-value
Lichen Network Similarity	3.5821	0.4130	0.0537	
Average Mutual Information	3.5235	0.3101	0.0254	
Centralization	4.0444	0.3305	0.0184	
Centralization In-Degree	4.4812	0.3487	0.0142	
Centralization Out-Degree	3.8615	0.3193	0.0205	
Centralization In-Degree (positive)	3.9852	0.3309	0.0190	
Centralization In-Degree (negative)	0.3304	0.1057	0.2508	
Centralization Out-Degree (positive)	3.5585	0.3119	0.0248	
Centralization Out-Degree (negative)	0.0862	0.0513	0.3446	
Number of Network Links (Degree)	3.5175	0.3156	0.0255	
Degree (positive)	3.6925	0.3242	0.0229	
Degree (negative)	0.0327	0.0318	0.3859	
Percent Lichen Cover	0.0000	0.0000	1.0000	
Lichen Species Diversity	0.0000	0.0000	0.4543	
Lichen Species Richness	0.0000	0.0000	0.4543	
Lichen Species Evenness	0.0000	0.0000	1.0000	
Percent Rough Bark	4.8526	0.3221	0.0113	
pH	0.0000	0.0000	1.0000	
Carbon-Nitrogen (CN) Ratio	0.0000	0.0000	1.0000	
Condensed Tannins (CT)	0.0007	0.0041	0.4439	
BR-L Residuals	0.0000	0.0000	1.0000	
BR-Cen Residuals	0.0000	0.0000	1.0000	

Table 2. Genotypic effects on tree traits and bark lichen.

	r	R2	estimate	SE	t	p-value
br_L	-0.34	0.11	-0.07	0.03	-2.13	0.04
br_Cen	-0.39	0.15	-0.00	0.00	-2.52	0.02
ct_L	0.34	0.11	0.57	0.27	2.13	0.04
ct_Cen	0.08	0.01	0.00	0.00	0.46	0.65
ph_L	0.08	0.01	0.67	1.41	0.48	0.64
ph_Cen	0.13	0.02	0.02	0.02	0.78	0.44
cn_L	0.06	0.00	50.19	145.84	0.34	0.73
cn_Cen	0.16	0.03	2.14	2.18	0.98	0.33

Table 3. Tests of the correlation between tree bark traits and lichen network structure

	df	SS	R2	F	p-value
geno	9.0000	44078.1327	0.5442	3.5821	0.0537
Residual	27.0000	36915.4605	0.4558		
Total	36.0000	80993.5932	1.0000		

Table 4. PERMANOVA Pseudo-F Table of lichen network similarity to genotype.

	Df	SumOfSqs	R2	F	Pr(>F)
BR	1.0000	21021.8765	0.2595	13.0299	0.0096
CT	1.0000	2349.3142	0.0290	1.4562	0.2016
pH	1.0000	2098.8999	0.0259	1.3010	0.2899
CN	1.0000	3896.1757	0.0481	2.4150	0.1890
Residual	32.0000	51627.3270	0.6374		
Total	36.0000	80993.5932	1.0000		

Table 5. PERMANOVA Pseudo-F Table of lichen network similarity response to bark traits.

lichen species	mean	statistic	H2	p-value
Positive				
<i>In-Degree</i>				
X. galericulata	0.2703	0	0	1
C. subdeflexa	0.8919	2.1926	0.2158	0.0595
L. spp.	0.4324	0	0	1
C. holocarpa	0.5946	3.6146	0.3241	0.024
X. montana	0.0541	0	0	0.4543
P. melanachra	0.1351	0	0	1
P. adscendens	0			
P. undulata	0.027	0	0	0.4543
R. sp.	0.1351	2.049	0.2613	0.0656
<i>Out-Degree</i>				
X. galericulata	0.027	0	0	0.4543
C. subdeflexa	0.6757	0	0	1
L. spp.	0.5946	0.0061	0.0126	0.4246
C. holocarpa	0.7027	3.1318	0.2981	0.0327
X. montana	0.0811	2.9228	0.3163	0.0375
P. melanachra	0.1351	0	0	1
P. adscendens	0			
P. undulata	0.027	0	0	0.4543
R. sp.	0.2973	0.1505	0.0612	0.3119
Negative				
<i>In-Degree</i>				
X. galericulata	0			
C. subdeflexa	0.1892	0	0	0.4543
L. spp.	0.1892	0.0015	0.0057	0.4398
C. holocarpa	0.1351	0	0	1
X. montana	0.027	0.0377	0.0394	0.3807
P. melanachra	0			
P. adscendens	0			
P. undulata	0			
R. sp.	0.1622	0	0	1
<i>Out-Degree</i>				
X. galericulata	0.2432	0	0	1
C. subdeflexa	0.4054	0	0	0.4543
L. spp.	0.027	0	0	0.4543
C. holocarpa	0.027	0	0	0.4543
X. montana	0			
P. melanachra	0			
P. adscendens	0			
P. undulata	0			
R. sp.	0			

Table 6. REML tests of the effect of tree genotype on lichen species centrality.

	BR	CT	pH	CN	PC	SR	SE	SD	L	Cen	Df	SumOfSqs	R2	F	Pr(>F)	
BR									-0.34	-0.39	geno	9.0000	1.5049	0.2001	0.7507	0.8878
CT									-0.34	0.34	Residual	27.0000	6.0143	0.7999		
pH											Total	36.0000	7.5193	1.0000		
CN																
PC																
SR									0.76	0.47						
SE									0.85	0.45						
SD										0.59	0.33					
L											0.88					
Cen																

Table 7. Matrix of correlations among tree traits, lichen community metrics and network metrics

Table 8. Pseudo-F Table of lichen community similarity PERMANOVA.

DRAFT

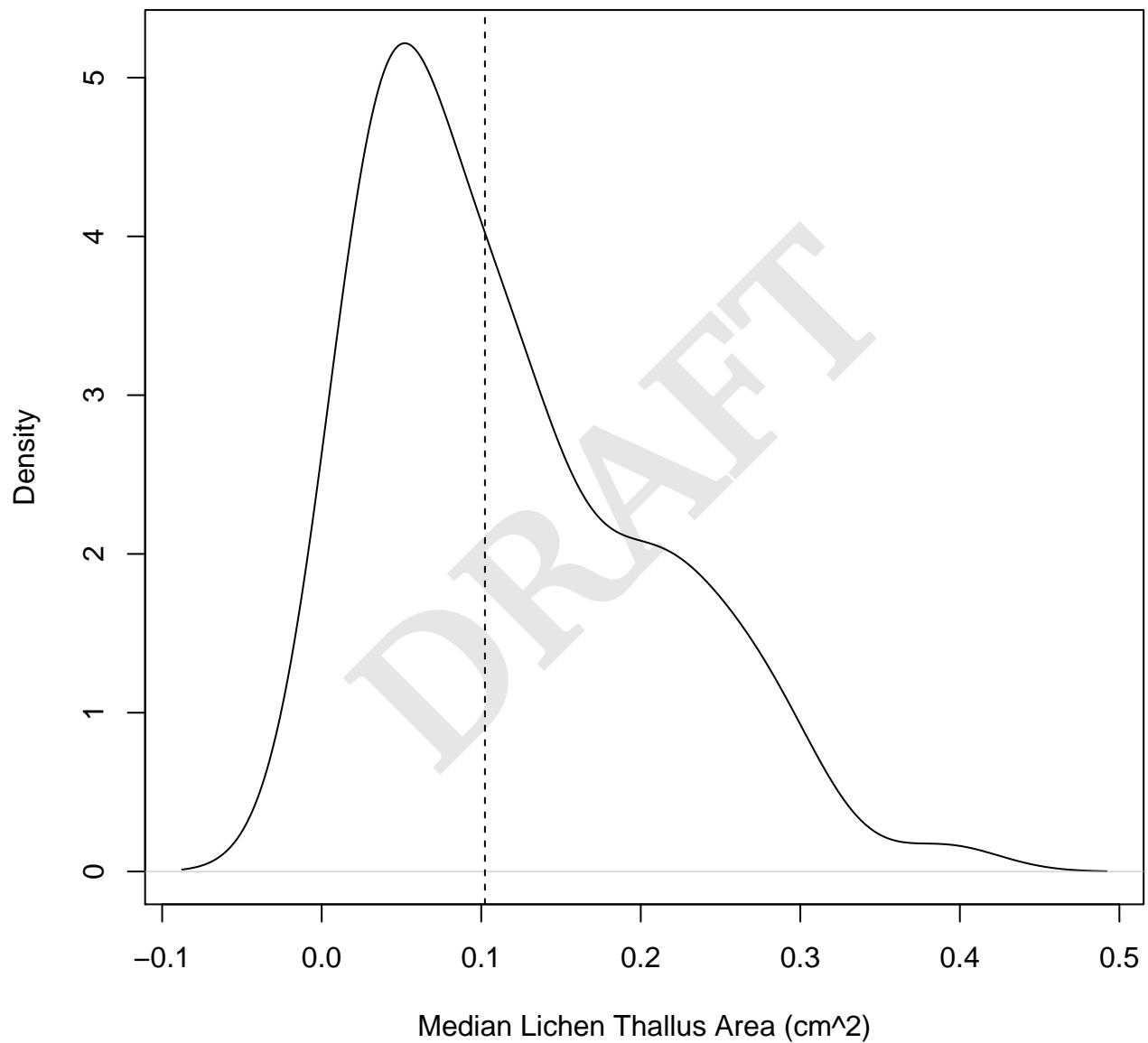


Fig. 1

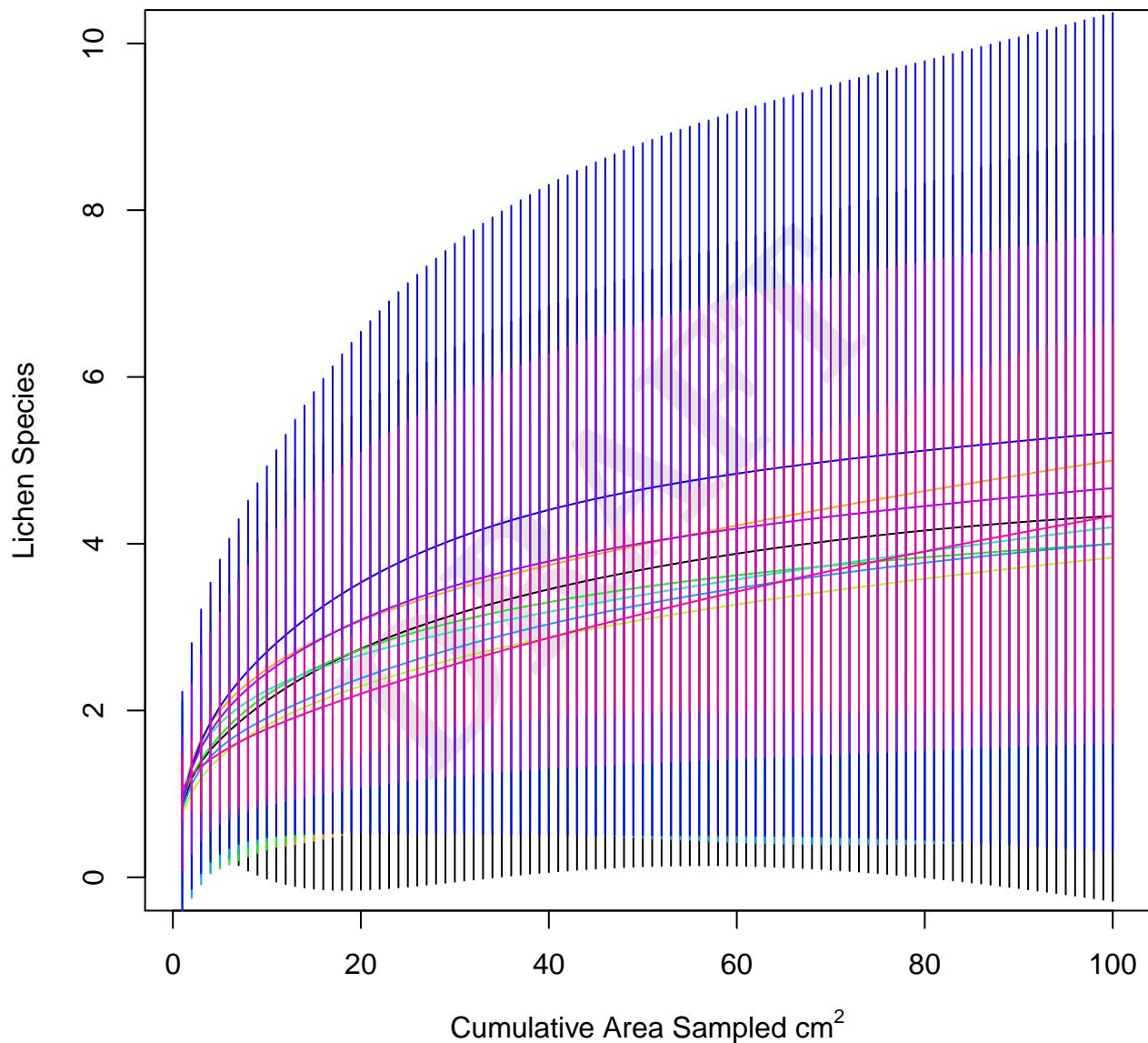


Fig. 2. Species area curve by genotype.

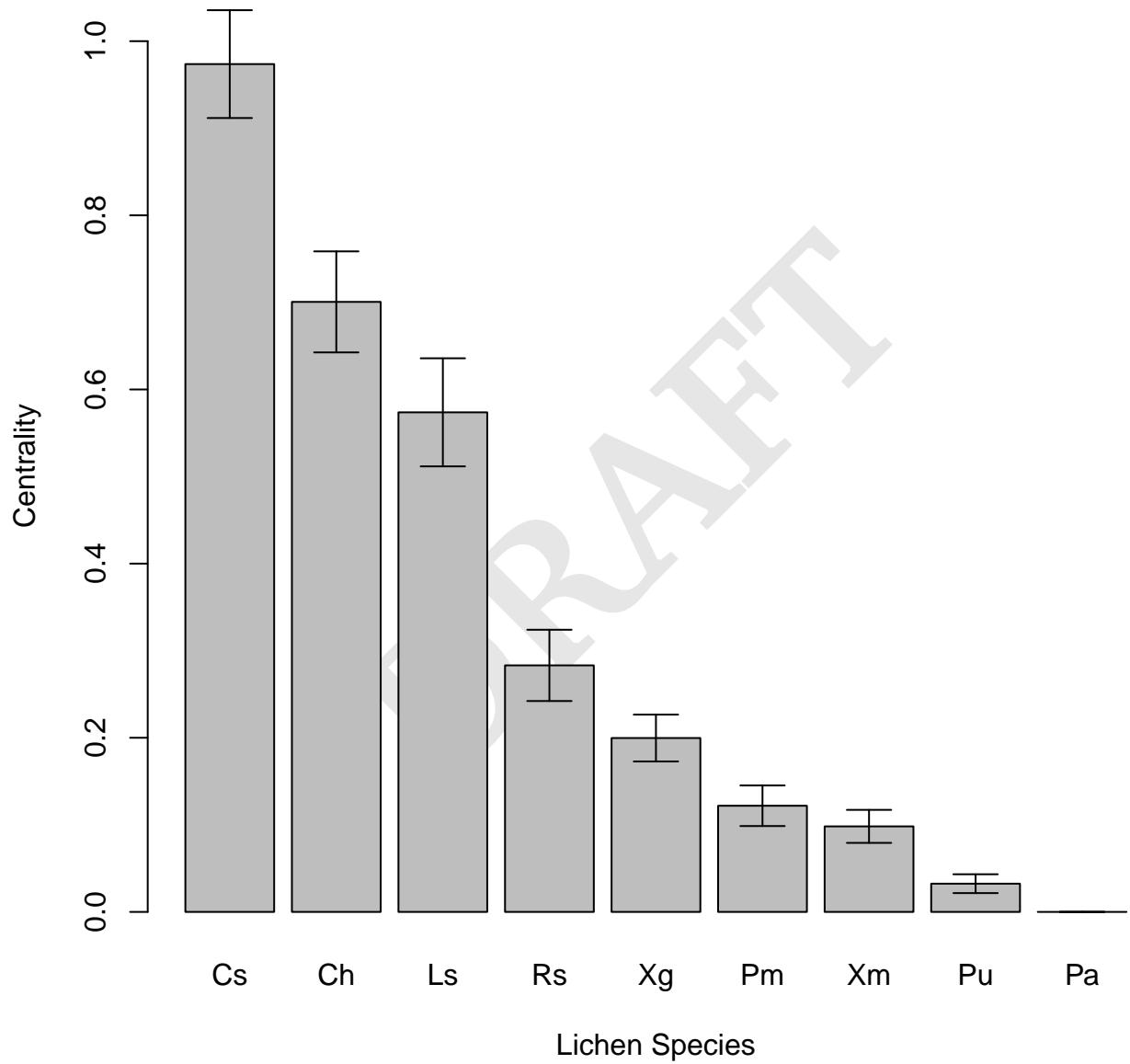


Fig. 3