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Testing a 'genes-to-ecosystems' approach to understanding aquatic–terrestrial linkages

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Abstract

A 'genes-to-ecosystems' approach has been proposed as a novel avenue for integrating the consequences of intraspecific genetic variation with the underlying genetic architecture of a species to shed light on the relationships among hierarchies of ecological organization (genes → individuals → communities → ecosystems). However, attempts to identify genes with major effect on the structure of communities and/or ecosystem processes have been limited and a comprehensive test of this approach has yet to emerge. Here, we present an interdisciplinary field study that integrated a common garden containing different genotypes of a dominant, riparian tree, *Populus trichocarpa*, and aquatic mesocosms to determine how intraspecific variation in leaf litter alters both terrestrial and aquatic communities and ecosystem functioning. Moreover, we incorporate data from extensive trait screening and genome-wide association studies estimating the heritability and genes associated with litter characteristics. We found that tree genotypes varied considerably in the quality and production of leaf litter, which contributed to variation in phytoplankton abundances, as well as nutrient dynamics and light availability in aquatic mesocosms. These 'after-life' effects of litter from different genotypes were comparable to the responses of terrestrial communities associated with the living foliage. We found that multiple litter traits corresponding with aquatic community and ecosystem responses differed in their heritability. Moreover, the underlying genetic architecture of these traits was complex, and many genes contributed only a small proportion to phenotypic variation. Our results provide further evidence that genetic variation is a key component of aquatic–terrestrial linkages, but challenge the ability to predict community or ecosystem responses based on the actions of one or a few genes.

Keywords: aquatic-terrestrial linkages, community genetics, decomposition, genes-to-ecosystems, GWAS, mesocosms, *Populus trichocarpa*

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Introduction

Over the past decade in ecology, there has been a shift in focus from species-level differences to variation within species, with multiple research avenues seeking to incorporate the ecology of individuals (Whitham *et al.* 2006; Bolnick *et al.* 2011; Schoener 2011). Genetic variation has been established as an essential component of individual variation, with consequences that extend well beyond the population level (Whitham *et al.*

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2006; Johnson & Stinchcombe 2007; Bailey *et al.* 2009). For example, there is growing support from community genetics research in a variety of study systems that intraspecific genetic variation, particularly within dominant or foundation plant species, can have wide-ranging effects on associated species, including herbivores, predators and pathogens (Whitham *et al.* 2012), and ecosystem processes, such as primary productivity and nutrient cycling (Madritch & Hunter 2002; Schweitzer *et al.* 2005; Crutsinger *et al.* 2006). Despite strong patterns stemming from the effects of genetic variation, many fundamental questions remain about the underlying mechanisms. For example, phenotypic variation among different genotypes is ultimately what dictates ecological interactions and most community genetics studies have either failed to measure traits or have centred their attention on one or a few key traits (Hughes *et al.* 2008; Hersch-Green *et al.* 2011). Similarly, understanding the genetic basis of traits, including how heritable they are and the underlying genes responsible for phenotypic variation, remains poorly explored within a community genetics context.

A 'genes-to-ecosystems' approach has been proposed as a novel framework for coupling the links between genetic underpinnings of phenotypes and the ecological consequences of phenotypic variation (Whitham *et al.* 2006, 2008). Building upon prior studies in community genetics, a 'genes-to-ecosystems' approach posits that individual genes can affect key traits that scale up to impact higher levels of organization. Identifying the genes associated with phenotypic variation in these traits should therefore facilitate a better understanding of how communities are assembled or ecosystems function (Whitham *et al.* 2008; Wymore *et al.* 2011). For example, the presence of allelic variation or shifts in allele frequency might predict the species composition of associated communities or perhaps how energy flows through a food web (Matthews *et al.* 2011; Farkas *et al.* 2013). While this reductionist approach is appealing, tests of a 'genes-to-ecosystems' approach have been limited. Studies have focused primarily on variation among different genotypes, levels of genotypic richness or across hybrid zones (Crutsinger *et al.* 2006; Hughes *et al.* 2008; Bailey *et al.* 2009; Whitham *et al.* 2012) and not on variation within individual genes. This is likely due to considerable costs and challenges remaining in disentangling genotypic to phenotypic pathways (Ingvarsson & Street 2011), let alone attempts to identify genes of major effect for an ecosystem process. An obvious method for testing a 'genes-to-ecosystems' approach would be to select simple Mendelian traits that vary under well-studied genetic pathways and then examine the effects on associated species or ecosystem processes. Undoubtedly, selecting candidate genes that explain a large proportion of the variation in a phenotype would reveal that the 'genes-to-ecosystems' approach is indeed possible under the right conditions. However, this method would fail to identify the relative importance of 'genes-to-ecosystems' linkages within the complexity of natural systems. A more thorough method would be to identify the traits that communities and ecosystem are responding to and then identify the genes that control these traits.

If genetic variation (considered here as variation among different genotypes) and individual genes are in fact linked to ecosystem processes, this raises further questions as to how far these effects might extend. Most studies of the ecological consequences of genetic and phenotypic variation have been confined to smaller components of ecosystems (Hughes *et al.* 2008; Whitham *et al.* 2012) and typically within terrestrial habitats (but see Hughes & Stachowicz 2004; LeRoy *et al.* 2006, 2007, 2012; Harmon *et al.* 2009). For example, it has been established for several decades now that different host-plant genotypes can support different communities of herbivores, with a resulting variability in their resistance/susceptibility to herbivore damage (Maddox & Root 1990; Hochwender & Fritz 2004). Yet, there is also strong evidence for aquatic–terrestrial linkages in which the movement of organisms and/or materials from one ecosystem can have major impacts on another (Polis *et al.* 2004; Knight *et al.* 2005). For example, seasonal pulses of leaf litter by terrestrial plants into streams, ponds and lakes (Marcarelli *et al.* 2011) have been shown to have a strong influence on trophic interactions and nutrient cycling in aquatic systems (Wallace *et al.* 1997; Gessner *et al.* 2010; Compson *et al.* 2013). A wide range of studies have focused on how variation in leaf litter inputs among plant species affects aquatic ecosystems (LeRoy & Marks 2006; Kominoski *et al.* 2011). The studies that have looked at the effects of genetic variation within species on aquatic ecosystems have used litterbags placed in streams and have concentrated exclusively on traits related to litter quality, such as secondary chemistry, ignoring genetic variation in other traits that might influence aquatic–terrestrial interactions (LeRoy *et al.* 2006, 2007, 2012; Marks *et al.* 2009; Jackrel & Wootton 2013).

In this study, we combined a common garden containing different genotypes of a dominant riparian tree with an aquatic mesocosm (1136-L cattle tanks) experiment and results from two existing data sets of extensive trait screening and genome-wide association studies (GWAS) (McKown *et al.* 2014a,b). The overarching goals of this research were (i) to understand the consequences of intraspecific variation in terrestrial leaf litter inputs for aquatic communities and ecosystem processes and how they compare to terrestrial

responses and (ii) to determine the specific host-plant traits that contributed most to variation in the aquatic community and relate the heritability of these traits and the underlying genetic architecture to ecological responses. By integrating multiple avenues, we explore the feasibility of a 'genes-to-ecosystems' approach through connecting a number of variable traits within a dominant species that influence ecosystem functioning, linking this to how many genes are associated with phenotypic variation in each trait, and partitioning the amount of phenotypic variation explained by individual genes. By addressing the genetic complexity of aquatic–terrestrial linkages in our study, we hope to provide a more detailed understanding of the causes and consequences of intraspecific genetic variation, as well as a thorough test of the practicality of a 'genes-to-ecosystems' approach.

Materials and methods

Study system

We conducted this research using wild accessions of black cottonwood (*Populus balsamifera* L. ssp. *trichocarpa* (Torr. & A. Gray ex Hook.); hereafter referred to as *Populus trichocarpa*). *Populus trichocarpa* is a native, dominant tree within riparian ecosystems in western North America, ranging from California to southern Alaska, is wind-pollinated and produces small, wind-dispersed seeds (Farrar 1995). Levels of genetic differentiation between *P. trichocarpa* populations are low to moderate (Wegrzyn *et al.* 2010; Slavov *et al.* 2012) but populations maintain high levels of heritable phenotypic differentiation (Weber *et al.* 1985; Xie *et al.* 2009), a pattern that suggests adaptation to local environmental conditions. With a fully sequenced genome, *P. trichocarpa* is also a model organism for understanding the genetic basis of phenotypic variability (Cronk 2005; Tuskan *et al.* 2006; Geraldes *et al.* 2013).

Propagation of tree genotypes

In March 2012, we selected five genotypes of *P. trichocarpa* from a pool of 461 accessions that originated from 136 localities throughout much of the native *P. trichocarpa* range (Xie *et al.* 2009; Fig. S1, Supporting information). All 461 genotypes had been growing in a common garden in Totem Field at the University of British Columbia in Vancouver, BC, since 2008 as part of an extensive genome-wide association study (McKown *et al.* 2014b). In the Totem Field common garden, trees were planted at 1.5 × 1.5 m spacing in a 40 × 54 m area with 4–20 replicates of each clone distributed in a fully randomized complete block design. Full methodological details of this common garden are outlined in McKown *et al.* (2013). Phenotypic trait data from this collection were used to calculate trait heritabilities and perform genome-wide associations (see below; McKown *et al.* 2014a,b). The five genotypes were selected to be equally related (average genetic distance = 0.326, range 0.317–0.334, see Fig. S2, Supporting information for further relatedness details) and representative of trait variation (height, growth rate, phenology) within southern BC localities (Latitude range: 49–52°N; Fig. S1, Supporting information), although they were chosen randomly within these prerequisites.

We propagated clones of each of the five selected genotypes using dormant whips collected from a single individual of each genotype that had been growing in the common garden at Totem Field since 2008. We then planted 20 cm cuttings from these whips in 'cone-tainers' containing a standard potting mix and placed them in a common greenhouse environment (25 °C) where they were watered as needed, fertilized with Osmocote (15:20:25; Scotts Miracle-Gro Co., Marysville, OH, USA) and randomized weekly in their location on greenhouse benches. After 10 weeks, we transplanted trees into individual 94.6-L plastic nursery containers containing a standard mixed topsoil (Premium Triple Mix topsoil; Acme Landfill and Peat Ltd, New Westminster, BC, Canada). Trees were fertilized once more after transplanting and watered as needed throughout the duration of the experiment.

Tree common garden × aquatic mesocosms

In June 2012, each *P. trichocarpa* genotype was placed in monoculture around experimental aquatic mesocosms with three replicate trees per mesocosm and 12 replicate mesocosms per genotype (180 trees, 60 mesocosms total) (Fig. S3, Supporting information). Mesocosms were constructed from 1136-L Rubbermaid cattle tanks (2 m in diameter, 1 m in depth). Tanks were randomly assigned in a 30 × 100 m grid with 3 m spacing on the campus of the University of British Columbia. Each tank was placed on a 3 × 3 m square of heavy-duty weed cloth to prevent vegetation growth in the immediate area and was filled with well water on 18–20 June 2012.

To establish an initial aquatic community, we added 11.33 kg of sterilized play sand to each tank, which was allowed to settle for 1 week creating ~1-cm sediment layer. We then inoculated each tank with phytoplankton and zooplankton using a plankton net with a cod end (80-cm net, 64-μm mesh; Dynamic Aqua-supply Ltd., Surrey, BC, Canada) and sampling five different nearby experimental ponds that contained a community dominated by small-bodied and large-bodied zooplankton

(e.g. calanoid copepods and *Daphnia* sp.). Plankton from all five ponds were homogenized and diluted. Two litres of water containing plankton were then added to each mesocosm. Next, we collected benthic mud from a nearby shallow lake (Browning Lake, Squamish, BC, Canada) and strained it through a 2.5-mm-diameter mesh sieve to remove any large detritus and benthic invertebrates. One litre of this mud was then added to each tank to inoculate a microbial community and add propagules of benthic and pelagic organisms. Finally, we added 1.23 g $NaNO_3$ and 0.09 g NaH_2PO_4 to each tank to boost initial primary production. Supplemental well water was added throughout the summer to compensate for evaporation, and mesocosms were left uncovered to allow for the natural colonization of other aquatic invertebrates.

At the end of September 2012, we bagged all *P. trichocarpa* individuals with vineyard netting (15 mm diameter, Smart Net Systems, Comox, BC, Canada) staked with a 3-m bamboo pole. From October–December, fallen leaf litter was collected weekly from under each tree, air-dried for 48 h and weighed, and 75% was deposited into mesocosms. The remaining 25% was used to estimate litter nutrient content and rates of decomposition. A portion of this litter was also oven-dried each week to correct final litter mass estimates for variation in initial leaf moisture content. At the end of December, when most of the leaves had fallen, the remaining leaves were collected from trees by hand, weighed and deposited in mesocosms. The portion of the study presented here ran for 16 weeks (October 2012–January 2013).

We note that mesocosms provide a useful tool for field studies, allowing a greater degree of realism compared to the laboratory setting but more control and replication than naturally occurring ponds (Odum 1984). This experimental setup was meant to mimic pools or ponds in the riparian ecosystems of British Columbia in which *P. trichocarpa* occurs as a dominant species. In this case, a small pond might receive the majority of its litter inputs from an adjacent tree. Cumulative litter inputs from the three *P. trichocarpa* individuals in this experiment ranged from 24 to 180 g DW per mesocosm (see results), which is a conservative estimate of litter inputs per m^2 from a full-sized *Populus* tree (Cotrufo *et al.* 2005, Meiresonne *et al.* 2007) and at the lower end of the range of litter inputs used in other aquatic mesocosm studies (e.g. Werner & Anholt 1996; Rubbo & Kiesecker 2004). We observed the natural colonization of a diverse aquatic community (see community-level responses) throughout the course of the experiment, suggesting the mesocosms represented a suitable natural aquatic habitat. Tree height ranged from 1 to 2 m by the end of the growing season and did not extend over the mesocosms at this point. Therefore, differences in shading among *P. trichocarpa* genotypes were not incorporated in this particular study.

Measuring individual-, community- and ecosystem-level responses to genetic variation

Individual trees. We measured a variety of *P. trichocarpa* traits associated with leaf litter, including tree height, the length of the three largest leaves per tree, weekly litter production and the timing of peak litter fall (when ~75% of all leaves had dropped from the tree canopy). We also measured litter nutrient (C, N, P) content and soluble condensed tannins (see Data S1, Supporting information for detailed methods). To examine how individual traits varied among *P. trichocarpa* genotypes, we used the average trait values for the three trees surrounding each tank and separate analysis of variance (ANOVA) models with genotype as the main factor.

Community. We measured several different aspects of aquatic community responses to *P. trichocarpa* genetic variation. For aquatic primary producers, we estimated the abundance of phytoplankton (chlorophyll-a) in each mesocosm in 3- to 4-week intervals three times throughout the experiment. For aquatic consumers, we measured zooplankton abundance and biomass, as well as benthic invertebrate richness, abundance, composition and total biomass. Zooplankton and benthic invertebrates were measured at a single time point during peak litter fall (see Data S1, Supporting information for detailed methods). We also examined mayflies specifically, as they were the most abundant aquatic macroinvertebrate. We used a repeated-measures ANOVA to test for the effects of *P. trichocarpa* genotypes on phytoplankton abundance using individual tanks as the unit of replication and time as the repeated measure. We used separate one-way ANOVA models to examine how zooplankton abundance and biomass, as well as benthic invertebrate richness, abundance and biomass, varied among *P. trichocarpa* genotypes. To examine whether benthic invertebrate community composition differed among *P. trichocarpa* genotypes, we calculated Bray–Curtis dissimilarity indices using relative abundance of each species and used analysis of similarity (ANOSIM; 999 restarts) to test for differences among tree genotypes. ANOSIM is analogous to an ANOVA on community dissimilarity values (PRIMER version 6). The generated Global R-statistic is a relative measure of separation between groups. A value of 0 indicates there is complete overlap in the community composition between groups, while a value of 1 indicates that there is no overlap (Clarke & Gorley 2006).

Ecosystem. We measured most aquatic ecosystem responses at regular intervals (three times at 3–4 weeks

intervals) throughout the experiment (see Data S1, Supporting information for additional methods). Responses included respiration, net primary productivity (NPP) and gross primary productivity (GPP). We also measured nutrient availability in the water, including soluble reactive phosphorous (SRP), dissolved organic carbon (DOC), ammonium (NH_4^+) and nitrate (NO_3^-). Litter decomposition was measured during peak litter production as mass lost from litterbags 1 month after being placed in mesocosms. Finally, we measured the light availability in mesocosms (hereafter referred to as 'light extinction') as the amount of photosynthetically active radiation (PAR) extinguished per centimetre of water (($PAR_{surface} - PAR_{depth}$)/measured depth) at the end point of the study (January 2014; see Data S1, Supporting information for detailed methods). We used separate repeated-measures ANOVA to test for differences among *P. trichocarpa* genotypes in terms of respiration, NPP, GPP, SRP, DOC, NH_4^+ and NO_3^- and separate one-way ANOVA models to examine differences in litter mass loss and light extinction. Data were log- or square-root transformed as needed to improve normality and reduce heteroscedasticity. For clarity, we show the untransformed values in all figures. We used Bonferroni's method to correct for multiple comparisons within individual- ($\alpha = 0.05/9$), community- ($\alpha = 0.05/6$) and ecosystem-level ($\alpha = 0.05/9$) analyses. We acknowledge that these corrections are conservative and likely inflated the probability of committing type II errors (Gotelli & Ellison 2004). Consequently, we treated each group of analyses (individual, community, ecosystem) as independent comparisons and corrected each separately.

Comparing aquatic and terrestrial responses to P. trichocarpa genetic variation

In order to compare the magnitude of the direct effects of *P. trichocarpa* genotype associated with living plant tissue to the 'after-life' effects associated with leaf litter, we sampled the foliar arthropod community and measured levels of herbivory on *P. trichocarpa* leaves during the following growing season. To sample foliar arthropods, we vacuumed the entire crown of each tree surrounding the mesocosms in June of 2013 (6 months after final litter additions to mesocosms occurred) using a modified leaf blower/vacuum (Craftsman 25 cc 2-cycle; Sears Holding Corporation, Hoffman Estates, IL, USA) with a fine insect net attached. We returned all samples to the laboratory where we sorted arthropods and identified them to species or morphospecies. We then scored leaf area removed by herbivores on ten leaves of each tree, starting with the first full-sized leaf at the top of the plant and measuring every other leaf up to ten. Leaves were scored as damage categories based on percentage leaf area removed (0%, 1–5%, 5–10%, 10–20%, 20–30%,… 90–100%). We estimated total arthropod richness and abundance by taking the average of the three trees surrounding each mesocosm. We also focused on the abundance of sawflies, the dominant species on the foliage, to compare with mayflies in the aquatic system. These data were not normally distributed, even after standard transformations. Therefore, we used separate generalized linear models using a Poisson distribution with a log link function to examine how total arthropod richness, arthropod abundance, sawfly abundance and herbivory damage varied among *P. trichocarpa* genotypes. Finally, we calculated the standard effect sizes (Cohen's *d*) of *P trichocarpa* genotype on terrestrial (total arthropod richness, total arthropod abundance, sawfly abundance) and aquatic (total invertebrate richness, total invertebrate abundance, mayfly abundance) communities. We also examined the effect sizes on aquatic and terrestrial leaf 'consumption' by comparing leaf damage due to herbivory with litter decomposition that occurred in litterbags in the mesocosms (detailed in ecosystem methods above and Data S1, Supporting information). Cohen's *d* was calculated as the difference between the means of the *P. trichocarpa* genotypes with the highest and lowest values for each response variable in each subsystem (aquatic vs. terrestrial) and then divided by the pooled standard deviation.

Populus trichocarpa traits accounting for community and ecosystem responses

To assess which *P. trichocarpa* litter characteristics explained community and ecosystems responses in aquatic mesocosms, we applied both least angle regression analyses (Efron *et al.* 2004) and path analysis (Shipley 2002) using R (R Core Team 2013). Least angle regressions are similar to stepwise regression in that they select the most parsimonious model, but are a more robust algorithm that overcomes some of the shortcomings of stepwise regressions (Hesterberg *et al.* 2008). Prior to these analyses, we first assessed multicollinearity among plant traits (averaged for the three trees per tank). Only leaf size and cumulative litter inputs were strongly correlated among mesocosms ($r = 0.72$, d.f. = 60, $P < 0.001$), and we retained cumulative litter inputs as an explanatory variable. To reduce the number of analyses, we focused on the aspects of the community (phytoplankton, zooplankton, mayflies) and ecosystem (decomposition, SRP, light extinction) that varied among mesocosms in relation to differences due to tree genotype and the sampling period (if repeated sampling occurred) where the largest differences among

genotypes occurred. Traits included cumulative litter production, timing of peak litter fall, nutrient content (C, N, P), condensed tannin content, litter mass loss and tree height. We also included mesocosm-level community and ecosystem parameters in the analyses to allow for indirect interactive effects of *P. trichocarpa* traits (e.g. litter nutrient content and zooplankton together might best explain phytoplankton). To understand which variables were most important in both community and ecosystem-level responses in mesocosms, we performed least angled regressions on ecosystem-level responses where we included both trait and community variables and on community-level responses where we included only plant trait variables. These regressions tested for only direct additive effects variables on ecosystem and community responses.

Next, we constructed a path model to better understand the direct and indirect relationships among plant traits, community responses and ecosystem responses in mesocosms. We included mesocosm-level community and ecosystem parameters in the analyses to allow for indirect effects of *P. trichocarpa* traits. Previous work on leaf litter subsidies in aquatic systems has suggested that carbon and tannins are negatively correlated with decomposition (Cotrufo *et al.* 2005) and therefore slow nutrient subsidies from litter. Phosphorous, nitrogen and the timing of subsidies have all been shown to influence productivity in aquatic ecosystems (Schindler 1977; Nowlin *et al.* 2008). Our path model built on these relationships, with litter traits having direct impacts on the plankton community, which in turn has impacts on water spectral properties (PAR) and decomposition. We considered two potential path models. Both examined links between litter traits (litter P, N and C content, condensed tannins, cumulative litter production, peak litter timing), community variables (phytoplankton and zooplankton abundances) and ecosystem variables (light extinction, litter decomposition). The second model allowed for the potential of direct links between traits and ecosystem responses by allowing all possible connections between variables (e.g. P can directly effect light extinction). We considered this model for light extinction and decomposition rate, but not for SRP, as this would have resulted in overfitting due to small sample size. Due to the cost of analyses, only a third (21 tanks, or ~5 per genotype) were sampled for SRP, whereas we sampled more tanks for other variables (see Table 1 for sample sizes). To determine whether there were direct or indirect effects, we calculated AIC for each model. We fit both models with the same procedure. First, we tested for the assumption of multivariate normality of the data with multivariate Shapiro test and found it to be non-normal (Shipley 2002). To account for this non-normality, we used the Satorra-Bentler corrected χ^2 statistic (Satorra 1990; Iriondo *et al.* 2003). The χ^2 statistic tests whether or not there is good fit between the model covariance structure and that of the data (a nonsignificant result indicates a good fit). We also assessed fit with the standardized root mean square residual (SRMR), where values of SRMR < 0.08 indicate an adequate fit (Hu & Bentler 1999). Once we fit each model, we compared them to each other and measured model fit with the χ^2 statistic and SRMR. We then estimated total R^2 for each community- and ecosystem-level variables and the partial R^2 of the individual- and community-level variables by calculating the sum of squares for each parameter and dividing that by the total sum of squares of the model.

Genetic complexity of P. trichocarpa traits

Broad-sense heritability. We include estimates of broad-sense trait heritability (H^2) that have been previously published for 448 *P. trichocarpa* accessions grown at the Totem Field common garden at UBC that were genotyped with a 34K SNP array (see McKown *et al.* 2014a for details on heritability calculations and for full trait heritability results). Briefly, H^2 calculations included trait values assessed by clonal replication of each accession with correction for population structure. Heritability values range from 0 to 1, with values closer to 1 interpreted as 'high' heritability indicating that a higher fraction of phenotypic variation is explained by underlying genetic variation and that traits are under stricter genetic control. In this study, we focused on 16 traits of interest from this larger data set that relate directly to the variation in amount, quality and timing of *P. trichocarpa* litter production observed among the five genotypes in the mesocosm experiment. Traits included five phenology traits (timing of bud break, leaf flush, bud set, canopy yellowing, leaf drop), one biomass trait (log height growth rate; log cm/day) and nine ecophysiological traits (maximum photosynthetic rate per unit mass [$A_{max/mass}$; μmol CO_2/g/s], leaf carbon:nitrogen content [C:N; mg/mg], chlorophyll content, leaf shape [length: width], leaf mass per area [LMA; mg/mm^2], carbon per leaf area [C_{area}; mg/mm^2], carbon per leaf mass [C_{mass}; mg/mg], nitrogen per leaf area [N_{area}; mg/mm^2], nitrogen per leaf mass [N_{mass}; mg/mg]). We also included H^2 estimates from condensed tannin content (μg/mg dry weight) (C. P. Constabel, unpublished data).

Association genetics. We used another existing data set from a genome-wide association study (GWAS) based on the same *P. trichocarpa* accessions to identify candidate genes for *P. trichocarpa* traits (McKown *et al.* 2014b). To date, this is the most extensive GWAS study

Table 1 Results from one-way and repeated-measures ANOVAs examining effects of genetic variation in *Populus trichocarpa* on variation in leaf litter traits (individual), as well as community- and ecosystem-level responses in adjacent aquatic mesocosms

	Effect	R^2	F	d.f.	P-value
Individual					
Cumulative litter	Genotype	0.70	33.26	4,57	**<0.0001**
Condensed tannins (%)	Genotype	0.69	19.32	4,35	**<0.0001**
P (%)	Genotype	0.46	11.70	4,55	**<0.0001**
N (%)	Genotype	0.45	10.35	4,51	**<0.0001**
C (%)	Genotype	0.03	0.43	4,51	0.788
C:N	Genotype	0.74	35.58	4,51	**<0.0001**
Plant height	Genotype	0.65	25.92	4,56	**<0.0001**
Leaf length	Genotype	0.70	33.45	4,56	**<0.0001**
Peak timing of litter fall	Genotype	0.46	12.18	4,56	**<0.0001**
Community					
Phytoplankton abundance	Genotype		2.99	4,56	**0.027**
	Time		9.02	2,55	**0.0004**
	Genotype × Time		1.67	8,110	0.113
Zooplankton abundance	Genotype	0.15	2.42	4,54	0.059
Zooplankton biomass	Genotype	0.08	1.16	4,52	0.338
Benthic insect abundance	Genotype	0.09	1.18	4,46	0.331
Benthic insect biomass	Genotype	0.15	2.22	4,49	0.080
Mayfly abundance	Genotype	0.26	3.61	4,41	**0.013**
Ecosystem					
Mass loss (%)	Genotype	0.41	8.69	4,52	**<0.0001**
Light extinction (PAR)	Genotype	0.31	6.18	4,54	**0.0004**
Gross primary productivity (GPP)	Genotype		2.17	4,55	0.084
	Time		45.67	2,54	**<0.0001**
	Genotype × Time		1.92	8,108	0.063
Net primary productivity (NPP)	Genotype		2.01	4,55	0.107
	Time		146.79	2,54	**<0.0001**
	Genotype × Time		1.78	8,108	0.088
Respiration (R = GPP–NPP)	Genotype		2.13	4,55	0.089
	Time		54.60	2,54	**<0.0001**
	Genotype × Time		1.81	8,108	0.084
Soluble reactive phosphorus	Genotype		6.40	4,24	**0.001**
	Time		4.85	2,23	**0.018**
	Genotype × Time		1.79	8,46	0.105
NH_4	Genotype		11.36	1,24	**0.003**
	Time		19.86	2,23	**<0.0001**
	Genotype × Time		0.98	8,46	0.465
NO_3	Genotype		0.48	4,24	0.748
	Time		3.14	2,23	0.063
	Genotype × Time		1.09	8,46	0.390
Dissolved organic carbon	Genotype		1.42	4,24	0.259
	Time		18.41	2,23	**<0.0001**
	Genotype × Time		0.48	8,46	0.864

Significant models indicated in bold.

of its kind in trees, using 29,354 filtered single-nucleotide polymorphisms (SNPs) representing 3,518 candidate genes. Significant SNP-trait associations were identified using a unified mixed model that accounted for population structure effects among the accessions and a strict Bonferroni-corrected threshold ($P < 1.7\times10^{-6}$). For this study, we focused on the GWAS results for 15 of the 16 traits of interest (tannins were not included in the study). To determine the genetic complexity of these traits, we present the total numbers of significant associations for each trait and the proportion of phenotypic variance captured by all significant SNPs (on a trait-by-trait basis). Our objective is to integrate the results of the larger GWAS study (McKown *et al.* 2014b) to further inform the genetic basis of litter characteristics and explore the potential

for individual genes that might account for community and ecosystem responses to litter inputs into aquatic mesocosms.

Results

What are the individual-, community- and ecosystem-level responses to genetic variation?

Individual trees. We observed considerable phenotypic variation among the five *P. trichocarpa* genotypes (Table 1). For example, mean maximum leaf length varied by 30%, while cumulative leaf litter production varied by over twofold among genotypes (Fig. 1a). There was also substantial variation in litter quality. Mean litter phosphorus (P) content varied by 30% (Fig. S4, Supporting information), leaf C:N varied by approximately twofold (Fig. 1b) and condensed tannins by ninefold (Table 1, Fig. 1c). Furthermore, the peak timing of litter fall (>75% canopy loss) varied by ~14 d among genotypes (Table 1).

Community. Aquatic community responses within the mesocosms containing different genotypes were relatively weak (Table 1). For primary producers, the amount of phytoplankton varied by up to 3.4-fold among ponds containing litter from different tree genotypes (Fig. 1d), but was not significant after Bonferroni correction. Within consumers, there was only a trend in the response of zooplankton abundance (Table 1, Fig. 1e). There were no overall differences in benthic invertebrate richness and abundance (Table 1), nor did community composition vary among tree genotypes (Global $R = 0.033$, d.f. = 58, $P = 0.136$). We did observe that the abundance of mayflies varied sixfold among the five *P. trichocarpa* genotypes (Table 1, Fig. 1f), although these differences were not significant when corrected for multiple comparisons.

As in the aquatic system, we observed differences in the terrestrial foliar arthropod community among the five *P. trichocarpa* genotypes. There was a 2.4-fold difference in foliar arthropod abundance (GLM, $\chi^2 = 80.47$, $P < 0.0001$), but there were no overall differences in arthropod richness (GLM, $\chi^2 = 3.25$, $P = 0.517$), nor did community composition vary among *P. trichocarpa* genotypes (Global $R = 0.032$, d.f. = 58, $P = 0.098$). Sawflies were the most abundant species on the foliage and varied by 2.8-fold among *P. trichocarpa* genotypes (GLM, $\chi^2 = 67.18$, $P < 0.0001$). Herbivore damage varied by 45% among *P. trichocarpa* genotypes ($F_{4,57} = 5.89$, $P < 0.0005$)(Fig. S4, Supporting information).

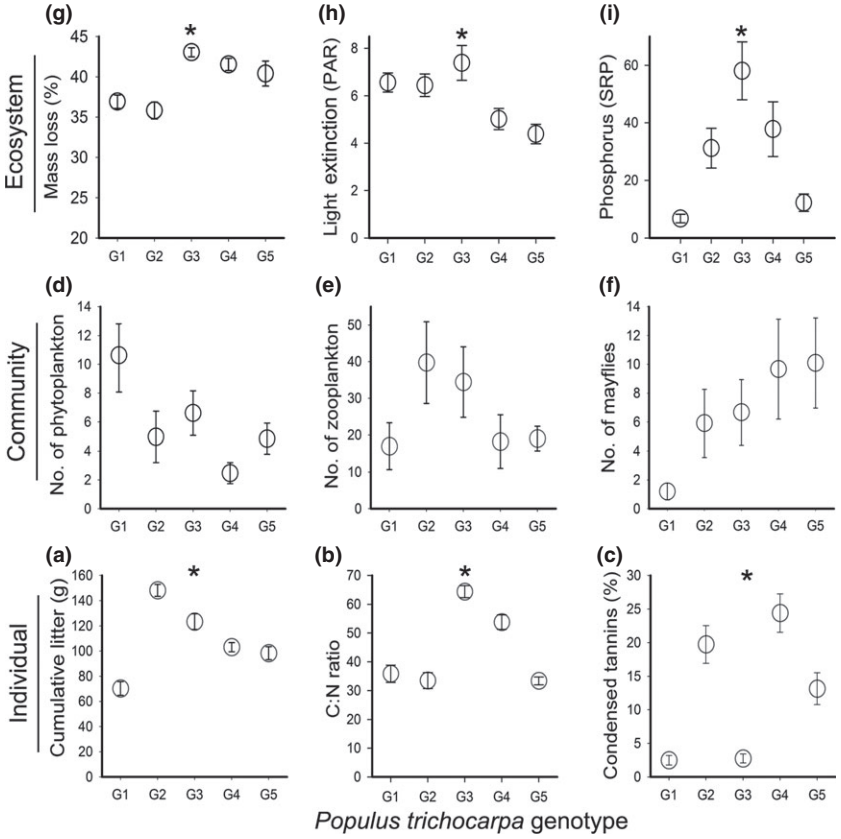

Fig. 1 Individual tree, aquatic community and aquatic ecosystem responses to variation among genotypes of black cottonwood, *Populus trichocarpa*. Individual tree responses included (a) cumulative litter inputs (g), (b) carbon: nitrogen ratio and (c) soluble condensed tannin content (%). Aquatic community-level responses included (d) phytoplankton abundance (measured as chlorophyll-a), as well as (e) zooplankton and (f) mayfly abundance. Aquatic ecosystem-level responses included (g) litter decomposition (% mass loss), (h) light extinction (loss of photosynthetically active radiation, PAR) and (i) soluble reactive phosphorus (SRP, μg/L) content in water. Circles represent means (±SE). *Significant responses after Bonferroni corrections for multiple comparisons.

Ecosystem. Genetic variation in *P. trichocarpa* leaf litter inputs resulted in much stronger shifts in aquatic ecosystem functioning compared to community-level responses (Table 1). *P. trichocarpa* genotypes varied in litter decomposition within litterbags placed in each tank (Fig. 1h), ranging from 35% to 43% of litter mass lost after a month. While there were no differences in primary productivity (GPP and NPP) or respiration, there were sizable differences in aquatic nutrient dynamics and the physical light environment among mesocosms receiving litter inputs from different tree genotypes. Light extinction varied by 69% (Fig. 1i), SRP by 8.6-fold (Fig. 1j) and NH_4^+ by 1.7-fold (Table 1). There were no significant differences in NO_3^- or DOC (Table 1).

How do the consequences of genetic variation compare in aquatic and terrestrial subsystems?

When we examined the effect sizes of *P. trichocarpa* genotype for aquatic and terrestrial responses, we observed fairly comparable effects for total richness, total abundance and the abundance of the most dominant species in both subsystems (Fig. 2). Moreover, we observed larger effects sizes for leaf consumption (i.e. decomposition) in aquatic systems compared to the terrestrial (i.e. herbivory) (Fig. 2).

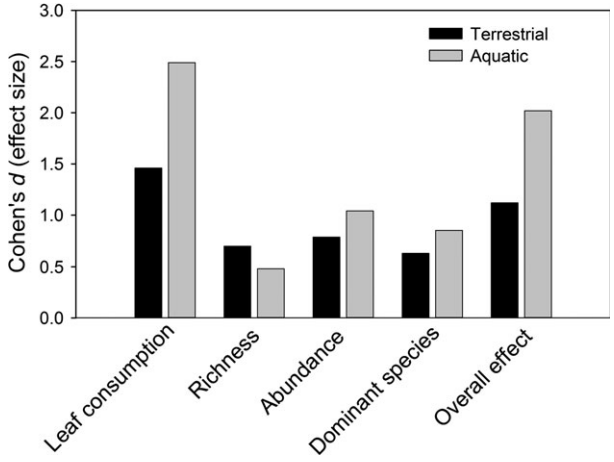

Fig. 2 Standard effect sizes (Cohen's *d*) comparing aquatic and terrestrial responses to five different *Populus trichocarpa* genotypes. Responses included total richness and abundance for foliar arthropods and invertebrates in litterbags placed in aquatic mesocosms, the abundance of the most dominant species (terrestrial sawflies, aquatic mayflies) and leaf consumption (leaf herbivory, aquatic litter decomposition). The overall effect is the average effect size of all responses within each subsystem.

Which traits account for aquatic community and ecosystem responses?

Using least angled regressions, we found that no individual trait was responsible for all aquatic community and ecosystem responses, rather that these responses were associated with a number of different traits. For example, phytoplankton abundance was negatively correlated with litter tannin content, but positively correlated with litter C and C:N, which together explained 33% of the variation in phytoplankton (Table 2). By contrast, the abundances of zooplankton and mayflies were not significantly related to any traits that we measured. At the ecosystem level, we found that 30% of the variation in light extinction was explained by litter C content and phytoplankton abundance (Table 2), whereas five different variables explained 60% of the variance in SRP (Table 2). Of these, three (cumulative litter inputs, zooplankton abundance, litter C:N) explained the majority of this variation. Litter decomposition was not significantly related to any traits included in our analyses.

Using path analysis, we found an indirect causal pathway between genetic variation in *P. trichocarpa* traits and ecosystem responses mediated by variation in the aquatic community (Fig. 3). When we compared this model to one with a direct link between litter C content and light extinction, we found that link was not supported ($\Delta AIC = 10$ between models), indicating the links between C and light extinction were mediated by phytoplankton abundance. This model was a good fit for the data with a Satorra-Bentler corrected χ^2 value of 0.168 ($P < 0.05$) and a SRMR of 0.065 (<0.08, indicating adequate fit; cf. Hu & Bentler 1999). Specifically, leaf litter C content ($P < 0.05$, partial $r^2 = 15\%$, standardized coefficient = 0.448) and tannin content ($P < 0.05$, partial $r^2 = 12\%$, standardized coefficient = −0.33) both caused an increase or decrease (respectively) in phytoplankton abundance ($r^2 = 30\%$). In turn, as phytoplankton abundance increased ($P < 0.05$, $r^2 = 17\%$), there was a corresponding increase in light extinction (whole model $P = 0.168$). We found no significant relationships between any plant traits that were measured and zooplankton abundance or decomposition rate, which corresponded with results from the least angled regressions model selection.

What are the genetic underpinnings of P. trichocarpa leaf litter traits?

When we examined broad-sense heritability values (H^2) for traits related to litter inputs (see Materials and Methods), we found that there was a substantial range in the H^2 estimates among traits (Table 3). Phenology traits such as timing of bud break, leaf flush, bud set,

Table 2 Results from least angled regression analyses predicting community and ecosystem responses to trait variation in *Populus trichocarpa* litter inputs from individuals growing adjacent to mesocosms. Responses with no significant predictors are included for informative purposes

Dependent variable	Independent variable	Parameter	Partial r^2	Model r^2	F	P	N
Phytoplankton	Tannins	−0.13	0.10	0.10	4.82	0.030	37
	Carbon	2.25	0.14	0.24	7.23	0.010	
	C:N	0.09	0.09	0.33	4.62	0.040	
Zooplankton	—	—	—	—	—	—	—
Mayfly	—	—	—	—	—	—	—
Light extinction	Carbon	0.39	0.14	0.14	5.64	0.020	32
	Phytoplankton	0.17	0.16	0.30	6.49	0.016	
Decomposition	—	—	—	—	—	—	—
Soluble reactive phosphorus	Cumulative litter	0.51	0.23	0.23	8.45	0.010	21
	C:N	0.34	0.11	0.34	4.05	0.060	
	Tree height	−0.16	0.08	0.42	3.06	0.100	
	Zooplankton	−9.25	0.14	0.56	5.10	0.040	
	Mayfly abundance	5.98	0.04	0.60	1.53	0.230	

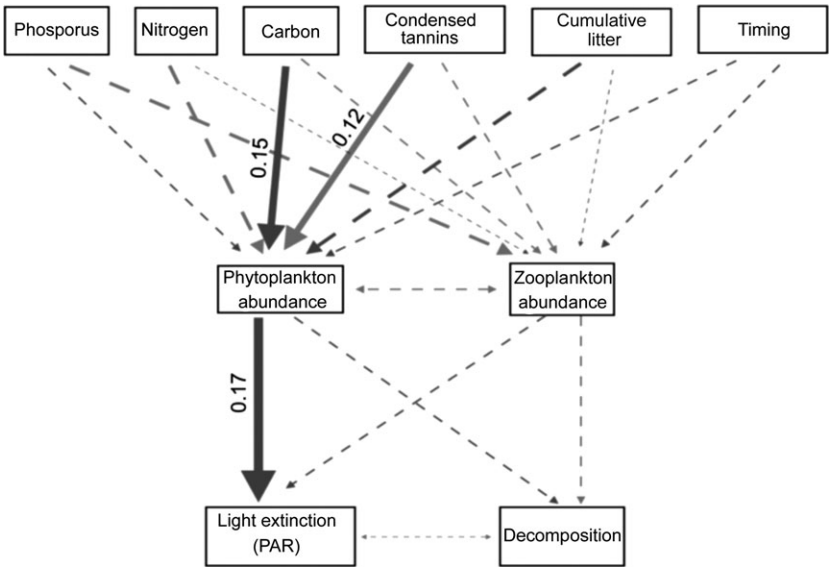

Fig. 3 Path diagram depicting the relationships between individual-, community- and ecosystem-level variables influenced by variation among genotypes of *Populus trichocarpa*, within a common garden integrated with aquatic mesocosms. Line width is proportional to standardized effect size, with lighter grey lines representing negative relationships and darker grey lines positive relationships. Significance ($P < 0.05$) is denoted by solid lines. The R^2 values correspond to the partial R^2 value for the path between two variables. Individual level variables carbon and condensed tannins accounted for 27% of the variation in phytoplankton abundance. Phytoplankton abundance in turn accounted for 17% of the variance in the ecosystem-level variable, photosynthetically active radiation (PAR).

canopy yellowing and leaf drop were highly heritable (H^2 = 0.56–0.88), indicating that the variation in the timing of litter inputs observed in the mesocosm experiment was largely under strong genetic control. Similarly, growth rate had moderately high heritability (H^2 = 0.47). By comparison, ecophysiological traits, such as $A_{max/mass}$, chlorophyll content of leaves, C and N content, LMA and leaf shape had comparably lower heritability values (H^2 = 0.17–0.37), indicating a stronger environmental influence for these traits. Notably, heritability of condensed leaf tannins (H^2 = 0.61; C. P. Constabel, unpublished data) was much higher compared to other traits related to litter quality.

Results from the GWAS indicated that the genetic architecture for *P. trichocarpa* traits was complex, with many genes involved in each trait, and that these genes were dispersed across the genome (McKown *et al.* 2014b; Table 3, Fig. 4). Moreover, the cumulative sum

Table 3 Broad-sense heritability estimates (H^2) for growth, ecophysiology and phenology traits affecting plant productivity measured across multiple years, total number of significant SNPs and genes uncovered using GWAS ($P < 1.7\times10^{-6}$) and cumulative R^2 explaining each trait in *Populus trichocarpa* accessions

Trait	Year	*n*	H^2*	SNPs/genes ($\alpha = 0.05$)†	SNP R^2 range†	Cumulative R^2†
Bud break (day)	2010	461	0.87‡	8/2	0.058–0.099	0.190
Bud break (day)	2011	461	0.89‡	3/1	0.058–0.10	0.121
Leaf flush (day)	2010	461	0.83‡	7/2	0.055–0.079	0.163
Leaf flush (day)	2011	461	0.88‡	7/2	0.059–0.093	0.175
Leaf flush (day)	2012	461	0.84	3/2	0.056–0.057	0.121
Bud set (day)	2008	457	0.66‡	54/41	0.026–0.049	0.290
Bud set (day)	2009	461	0.82‡	97/64	0.028–0.066	0.362
Bud set (day)	2010	461	0.73‡	92/64	0.019–0.047	0.260
Canopy yellowing, 100% (day)	2010	458	0.59‡	33/24	0.012–0.020	0.120
Leaf drop (day)	2008	455	0.58‡	62/51	0.024–0.055	0.270
Leaf drop (day)	2009	461	0.59‡	106/79	0.023–0.055	0.332
Leaf drop (day)	2010	461	0.63‡	55/41	0.012–0.022	0.148
Log height growth (log cm/day)	2009	460	0.47‡	5/4	0.040–0.045	0.153
$A_{max/mass}$ (μmol CO_2 g/s)	2009	455	0.17	2/2	0.056–0.057	0.084
C:N (mg/mg)	2009	454	0.21	1/1	0.069	0.0054
Chl_{summer} (CCI)	2009	414	0.31	6/5	0.058–0.082	0.115
Chl_{summer} (CCI)	2011	369	0.34	5/4	0.065–0.099	0.218
Leaf shape (length:width)	2009	461	0.37	6/5	0.060–0.078	0.181
LMA_{summer} (mg/mm^2)	2009	455	0.17	0	—	—
LMA_{summer} (mg/mm^2)	2010	369	0.27‡	0	—	—
LMA_{summer} (mg/mm^2)	2011	369	0.32‡	0	—	—
N_{area} (mg/mm^2)	2009	455	0.21	0	—	—
N_{mass} (mg/mg)	2009	455	0.21	6/5	0.058–0.064	0.169
C_{area} (mg/mm^2)	2009	455	0.18	0	—	—
C_{mass} (mg/mg)	2009	455	0.22	0	—	—
Tannins (μg/mg/DW)§,¶	2011	455	0.61‡	N/A	N/A	N/A

$A_{max/mass}$ = photosynthetic rate per unit dry mass; C:N = carbon:nitrogen; Chl = chlorophyll content; LMA = leaf mass per unit area; N = nitrogen; SNPs = single-nucleotide polymorphisms.
*Data from McKown *et al.* (2014a).
†Data from McKown *et al.* (2014b).
‡Data corrected for spatial trends within Totem Field.
§C. P. Constabel, unpublished data.
¶Data log-transformed for normality.

of SNP-trait associations (cumulative R^2) explained only a small proportion of the phenotypic variation (Table 3). For example, bud set in *P. trichocarpa* was highly heritable ($H^2 = 0.72$) and was associated with 172 genes with SNPs that individually explained 2–10% of the variation but cumulatively explained only 36% of the total trait variation. In other cases, the number of genes retrieved was lower (e.g. a single gene for C:N) but explained a low percentage of trait variance (0.5% of overall variability in C:N; Table 3). Similarly, condensed tannins had a relatively high H^2 estimate and preliminary results indicate a complex genetic architecture underlying trait variability (C. P. Constabel, unpublished data). While the GWAS implicated high genetic complexity for some traits of interest, other traits lacked sufficient genetic information. This was particularly the case for ecophysiology traits (e.g. LMA) that had low H^2 (and consequently higher trait plasticity) and few (or no known) genetic associations.

Discussion

Our results offer novel insight into the pathways by which genetic effects spill over the boundaries of aquatic and terrestrial ecosystems and the extent to which employing a 'genes-to-ecosystem' approach is feasible. We observed phenotypic variation among *P. trichocarpa* genotypes in a variety of traits associated with leaf litter subsidies to aquatic mesocosms, including traits related to tree phenology, growth and ecophysiology. In turn, this phenotypic variation in leaf litter had relatively weak effects on aquatic producers (phytoplankton) and

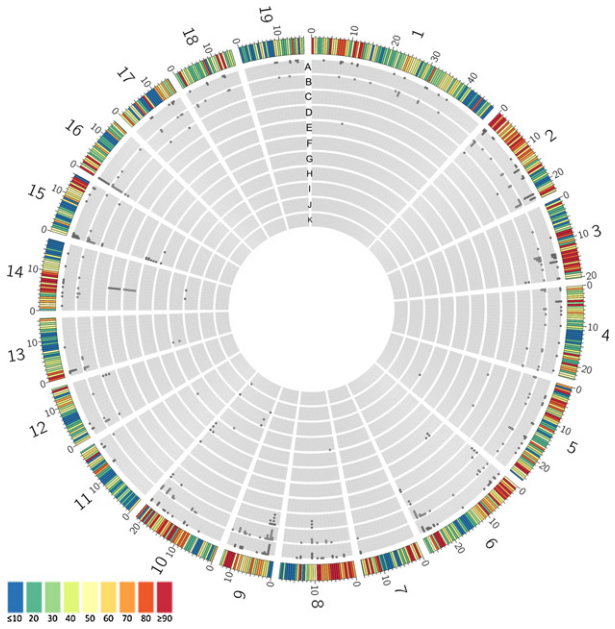

Fig. 4 Genomic distribution of single-nucleotide polymorphisms (SNPs) on the 34K SNP genotyping array and significant SNPs uncovered using genome-wide association study (GWAS) across 19 chromosomes in *Populus trichocarpa*. The outer ring shows the number of SNPs genotyped in each 0.5 Mbp of the *P. trichocarpa* genome (bottom legend depicts SNP densities). The genomic location and number of SNPs significantly associated with each trait is indicated as points on the inner rings. Traits categories represent included timing of bud set (ring A), leaf drop (B), 100% canopy yellowing (C), bud break (D) and leaf flush (E). They also included leaf chlorophyll content (F), nitrogen content per leaf area [N_{area}; mg/mm^2](G), leaf shape [length:width](H), tree growth rate [log cm/day](I), maximum photosynthetic rate per unit mass [$A_{max/mass}$; μmol $CO_2/g/s$](J) and carbon:nitrogen content [C:N; mg/mg](K).

consumers (zooplankton and mayflies) but stronger effects at the ecosystem level, driving litter decomposition, nutrient availability and light extinction within mesocosms. Yet, we found that different traits corresponded with different community and ecosystem responses. While tannins have been shown strong genetic effects within the *Populus* system (Whitham *et al.* 2006, 2008), we observed that variation in tree growth, litter nutrient content and productivity of litter inputs were also important factors explaining portions of the variation in aquatic community and ecosystem responses. These results suggest that the ecological influence of *P. trichocarpa* genetic variation is dispersed across a variety of important traits, as opposed to a single underlying mechanism. Moreover, we found that *P. trichocarpa* genotypes that input more carbon and fewer tannins into mesocosms had a positive relationship with phytoplankton abundance, which then led to a decline in the amount of available light (i.e. higher light extinction). As such, our findings illustrate that the links between tree phenotypic variation and aquatic ecosystem function can occur indirectly via community-level responses. We note that the primary genetic effect in this case stems from tannins, as C content did not vary significantly among *P. trichocarpa* clones.

To date, the bulk of studies on the community and ecosystem consequences of genetic variation have been performed using terrestrial plants and terrestrial communities (Bailey *et al.* 2009), with exceptions (e.g. Hughes & Stachowicz 2004; LeRoy *et al.* 2006, 2007, 2012; Stachowicz *et al.* 2013). We examined foliar arthropod responses to the different *P. triocharpa* genotypes and observed strong differences in arthropod abundance, notably sawflies, which corresponded with differences in herbivore damage among clones. Perhaps these results are not too surprising, given the range of other systems in which community responses to genetic variation have been observed (Whitham *et al.* 2012). What was surprising was that the effect sizes of genetic variation on aquatic invertebrates and decomposition were as strong as effects on foliage arthropods and herbivory on the leaves themselves. Therefore, our results indicate that intraspecific genetic variation can mirror the ecological effects of interspecific variation (Kominoski *et al.* 2010) for aquatic–terrestrial linkages and that the 'after-life' effects of leaf litter inputs from different genotypes into aquatic systems can be just as strong as the consequences associated with live foliage.

The few studies that have specifically addressed the role of terrestrial genetic variation in aquatic ecosystems have performed litterbag experiments in natural streams systems (LeRoy *et al.* 2006, 2007), as opposed to creating and assessing entire aquatic mesocosms. LeRoy *et al.* (2006, 2007) examined leaf litter from two *Populus* parental species (*P. fremontii*, *P. angustifolia*) and their hybrids and found differences both among and within parental species and hybrids driven primarily by variation in condensed tannins. The five *P. trichocarpa* genotypes used in our study were equally distantly related and represented a more conservative estimate of genetic variation compared to two distinct species and their hybrids, however, we still observed a considerable amount of variation in litter traits among *P. trichocarpa* genotypes.

We placed the five *P. trichocarpa* genotypes in our study into a broader context of a trait screening study based on the large collection of genotypes originating from across the *P. trichocarpa* range (McKown *et al.* 2014a,b). We note that our application of the heritability estimates from this study to our tested genotypes is within the context of many genotypes from a larger geographic area planted in a common garden and acknowledge the potential for mismatch in scale that

might inflate estimates of the ecological importance of genetic effects for a given locality (Tack *et al.* 2012). Still, we believe there are valid conclusions to be drawn about the genetic basis of *P. trichocarpa* traits. In particular, we found that there were substantial differences in the heritability values for different traits related to the timing, productivity and quality of *P. trichocarpa* leaf litter and variation in these traits would ultimately effect nutrient inputs into the riparian environment. For example, tree phenology, growth rate and condensed tannins were highly heritable (Table 3), suggesting that differences in the timing of litter production, amount of litter produced and litter quality should be attributed predominantly to underlying genetic factors. By contrast, many ecophysiological traits, such as the C:N content or chlorophyll content of leaves, had fairly low heritability values, suggesting that nongenetic factors, such as the local environment, will play an important and influential role in the nutritional quality of litter produced across different localities regardless of genotype.

Variation in trait heritability values could affect the predictability of ecological responses based solely on underlying genetic variation. The fact that community and ecosystem responses were related to multiple traits, such as the amount of litter, C:N content and tannins, rather than a single trait, compounds the issue. Accordingly, researchers can either limit themselves to ecological interactions that correspond exclusively with highly heritable traits (relying more on individual genetic variation) or they can incorporate the environment, as well as genotype × environment interactions into their studies. The latter addresses the multiple drivers of phenotypic variation and is of broader interest to ecologists that study the role of individual variation in shaping communities and ecosystems (Hughes *et al.* 2008; Bolnick *et al.* 2011; Schoener 2011; Violle *et al.* 2012). While there have been studies examining the relative influence of genetic variation and the environment (Madritch *et al.* 2006; Crutsinger *et al.* 2013), particularly in regards plant–insect interactions (Tack *et al.* 2012), many questions are left about the ecological consequences of genotype × environment interactions and extrapolating these effects to ecosystem functioning.

The extensive GWAS data available for *P. trichocarpa* (McKown *et al.* 2014b) allowed us to explore the genetic basis of traits that corresponded with leaf litter. We found that community and ecosystem responses were linked to traits that have a complex genetic basis (Table 3). This was particularly observed in growth rate, canopy yellowing and leaf drop, and N_{mass}. In each case, these key traits relating to litter were associated with multiple genes. For other key traits, GWAS failed to identify significant associations or retrieved only a small number of genes. For example, this was observed in C:N, which retrieved a single gene, but only explained 0.05% of the trait variation. Thus, while comprehensive, the GWAS results did not retrieve a large proportion of variation in traits associated with leaf litter and posits that further gene associations may be retrieved. For instance, each SNP association explained only a fraction of the phenotypic variation and considered together, the cumulative variation explained was less than 50% for any given trait. This missing variance (or lack of associations) may largely relate to genes not present on the SNP array or due to significant challenges of low power in detecting genes of small effect or rare alleles. For example, the strongest relationship between *P. trichocarpa* traits and an ecological response occurred between cumulative litter inputs and SRP where litter inputs explained 23% of the variation in SRP across mesocosms (Table 2). Although litter inputs were not measured in the association study, inputs were strongly predicted by leaf shape in our experiment ($r = 0.72$, $P < 0.001$). In this case, there were six significant SNP associations (5 genes), each individually explained 6–7% of the variation in leaf shape and cumulatively explained 7.2% (Table 3).

These findings are not unexpected and closely parallel patterns within *Eucalyptus globulus*, in which genetic variation has also been shown to have important ecological consequences (Barbour *et al.* 2009). In a smaller study of 195 SNPs from 24 candidate genes, Kulheim *et al.* (2011) found that *E. globulus* traits were also polygenic, with many small contributions (average of 2.95%; the largest effect explained only 6% of the phenotypic variation). Moreover, the proliferation of QTL studies and genome-wide sequence data has yielded a general consensus that most traits are polygenic with only a small fraction of phenotypic variability influenced by a single gene (Perfeito *et al.* 2007; Barrick *et al.* 2009). For example, DeWoody *et al.* (2013) used QTL analyses to identify genomic regions that correlated with insect herbivores associated with hybrid poplars (*P. trichocarpa* × *P. deltoides*) and found multiple loci dispersed across the genome that correlated with herbivores, with different loci identified depending on the time of the measurement within the growing season.

Nonetheless, our results strongly suggest that it is unlikely that a single gene (or genetic pathway) determines trait variation relating to leaf litter. As many studies have found that traits are largely polygenic, we expect that future studies on the underlying genetics of leaf litter traits may have similar results. This calls into question the feasibility of undertaking any true 'genes-to-ecosystems' approach when multiple genes may play a role in determining the phenotype of an important trait. Furthermore, while some traits were considered

highly heritable (i.e. more predictable across years and from parents to offspring) and thus potentially more attractive for ecological studies, we found that many important traits in our system had lower heritability and lacked this predictability due to a larger proportion of the phenotypic variance being explained by the environment. In this case, pursuing any cascading effects of suites of genes relating to these traits may be problematic when the trait itself is under less strict genetic control. Next steps for this research would be a full association study of community and ecosystem responses using the whole genome, as well as new experiments with more complex mixtures of poplar genotypes. Furthermore, we encourage additional tests of the 'genes-to-ecosystems' approach in other systems to determine the extent to which this approach might be useful, as well as lead to productive collaborations among researchers interested in the links between genes and phenotypes and those interested in the ecological consequences of phenotypic variation.

Conclusions

The overarching goals of our study were to relate the genetic basis of individual variation in leaf litter traits and the impacts on the flow of energy and nutrients between aquatic and terrestrial ecosystems. We observed high phenotypic variation among *P. trichocarpa* genotypes that had a variety of direct and indirect effects on the experimental mesocosms. That some important traits were highly heritable (e.g. phenology), while others were much less so (e.g. leaf nutrient content), suggests that more research is needed to partition genetic vs. environmental influences on aquatic–terrestrial linkages. In addition, we tested the 'genes-to-ecosystems' approach (Whitham *et al.* 2008; Wymore *et al.* 2011). We found that there are many genes associated with *P. trichocarpa* litter traits, and thus, the potential of linking the action of any single gene to an ecological response is very low. Yet, the complex genetic architecture of *P. trichocarpa* traits does not negate the ecological importance of intraspecific genetic variation in this system, as well as other systems out there (Whitham *et al.* 2006; Hughes *et al.* 2008; Bailey *et al.* 2009). Our study adds further support for the community genetics perspective that integrating genomics with community and ecosystem ecology can shed light on the relationships among hierarchies of ecological organization and reveal the mechanisms underlying the consequences of complex genetic variation.

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Data accessibility

Populus trichocarpa trait data, aquatic community and ecosystem data: Dryad doi:10.5061/dryad.1nq6m. Structural Equation Model data file and R script: Dryad doi:10.5061/dryad.1nq6m.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Map of localities where *Populus trichocarpa* genotypes were collected (closed 2 circles). Open circles represent the genotypes used in the mesocosm experiment.

Fig. S2 Unrooted neighbor—joining tree constructed using 24,083 SNPs with minor allele frequency higher than 0.1 and no missing data among the five *Populus trichocarpa* genotypes used in this study.

Fig. S3 Photo of randomized experimental array showing genotypes of black cottonwood, *Populus trichocarpa*, surrounding aquatic mesocosms (Photo credit: G. Crutsinger)

Fig. S4 Trait variation among genotypes of black cottonwood, *Populus trichocarpa*, including phosphorus (P) content in leaves, tree height, maximum leaf length, and herbivory (leaf area removed). Bars represent means (±SE).

Data S1 Methods.