

REPORT

Genetically based trait in a dominant tree affects ecosystem processes

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Abstract

Fundamental links between genes and ecosystem processes have remained elusive, although they have the potential to place ecosystem sciences within a genetic and evolutionary framework. Utilizing common gardens with cottonwood trees of known genotype, we found that the concentration of condensed tannins is genetically based and is the best predictor of ecosystem-level processes. Condensed tannin inputs from foliage explained 55–65% of the variation in soil net nitrogen (N) mineralization under both field and laboratory conditions. Alternative associations with litter lignin, soil moisture or soil temperature were relatively poor predictors of litter decomposition and net N mineralization. In contrast to the paradigm that the effects of genes are too diffuse to be important at the ecosystem-level, here we show that plant genes had strong, immediate effects on ecosystem function via a tight coupling of plant polyphenols to rates of nitrogen cycling.

Keywords

Condensed tannins, ecosystem ecology, extended phenotype, genetic diversity, leaf litter decomposition, nitrogen mineralization, plant hybridization, *Populus*.

Ecology Letters (2004) 7: 127–134

INTRODUCTION

Although it is well established that gene expression produces a phenotype that is expressed in an individual, much less understood is the effect of the genotype on community interactions and ecosystem processes (Whitham *et al.* 2003). Recent studies have begun to address the genetic determinants of ecosystem function at the phenotype level (Driebe & Whitham 2000; Treseder & Vitousek 2001; Madritch & Hunter 2002), although the specific genetic mechanisms are unknown. For example, Madritch & Hunter (2002) found that genetic distance between oak phenotypes was related to litter chemistry, which had subsequent effects on both carbon and nitrogen (N) fluxes in an oak forest. These studies are timely as research with many plant taxa suggests that there is a strong genetic basis for the variation in chemical expression in plants, irrespective of environmental influences (Nichols-Orians *et al.* 1993; Adler *et al.* 1995; Hwang & Lindroth 1997).

In a recent review, Hamilton *et al.* (2001) demonstrated that plant phylogenetic history, measured as broad-sense

heritability, more commonly explains secondary chemical concentrations in leaves than do environmental factors. The genetic component of plant secondary compound synthesis is becoming more appreciated as data indicate that genetics can explain from half to all of the variation in plant chemical concentrations. Because secondary compounds, such as polyphenols, are known to influence the quality of plant litter as a substrate for microbial decomposition (Hättenschwiler & Vitousek 2000; Hättenschwiler *et al.* 2003), we hypothesized that genetically based variation in the concentrations of these compounds could result in important extended phenotypes (i.e. expression of genes at levels higher than the individual or population) that could alter ecosystem function.

Plant hybrids, including oak, pine, eucalypts, iris, sunflowers and many others, have widespread distributions throughout temperate ecosystems (Arnold 1997). Natural hybridization results in high genetic diversity in many traits, including plant secondary chemistry (Orians 2000), and represents a critical pathway of speciation in many plant groups (Smith & Sytsma 1990; Carr 1995). *Populus* hybrid

zones represent a model system to examine relationships between plant genes and ecosystem processes as *Populus* species hybridize naturally, creating genetically recombinant populations ideal for localizing quantitative traits and tracing the introgression of specific alleles (Keim *et al.* 1989; Rieseberg & Buerkle 2002). Furthermore, *Populus* trees comprise a majority of the standing biomass of many western North American riparian habitats (Eckenwalder 1984a), making their effects on dependent communities and ecosystem function substantial. Our results suggest that the genetic basis for polyphenol production in *Populus* hybrids is tightly coupled to decomposition and nitrogen cycling, suggesting that intraspecific variation is an important regulator of ecosystem function.

MATERIALS AND METHODS

Leaf and litter quality

To compare the genetic component of leaf quality, we assessed chemical differences between *Populus* species (*Populus fremontii* L.; Fremont cottonwood and *P. angustifolia*, James; narrowleaf cottonwood) and their natural hybrids grown in the same environment and in the field. Pure stands of *P. fremontii* and *P. angustifolia* are found at low and high elevations, respectively, along rivers throughout the western United States of America (USA). At the middle elevations where their distributions overlap, these species freely hybridize to form a hybrid 'swarm' (Eckenwalder 1984 a,b). The hybrid zone is composed of F₁ hybrids and complex backcrosses, and both pure species, creating stands of trees with high genetic variation (Keim *et al.* 1989; Martinsen *et al.* 2001) and variable ecological properties (Whitham *et al.* 1999). The intersectional hybridization of these two species occurs in drainages throughout the western USA and represents uni-directional introgression (Keim *et al.* 1989) where the F₁ hybrids can only backcross with one parent (narrowleaf cottonwood) to create backcross generations.

We utilized a common garden planted in 1991 at the Ogden Nature Center in Ogden Utah, USA, to examine the genetic basis for leaf phytochemistry. The common garden is composed of trees that were propagated from local field trees of known genotype of both species, F₁ hybrids and a range of backcross hybrids, determined by restriction fragment length polymorphisms (RFLP) analyses (see Martinsen *et al.* 2001 for details) that were randomly planted within the site. The 35 species specific RFLP markers were used to accurately discriminate between the individual tree genotypes and determine hybrid status. We collected green leaves from 10 random genotypes of each species (*P. fremontii* and *P. angustifolia*) and cross type (F₁ and a range of backcross hybrid generations) from the common garden in

July 2000 ($n = 40$ genotypes total). The leaves were collected mid-growing season, flash-frozen on dry ice, lyophilized and stored in a -80 °C freezer until analysed chemically. Because the introgression occurs only in one direction (toward narrowleaf, we regressed (with a logistic model) the proportion of Fremont marker alleles for each genotype against the litter quality parameters (described below).

At leaf fall, in the same common garden in 2000, we collected and pooled leaf litter from 10 random genotypes of each species (*P. fremontii* and *P. angustifolia*) and cross type (F₁ and a range of backcross hybrid generations) to be used in the decomposition experiment. The litter was collected in mesh bags tied around large branches of trees of known genotype. We compared the concentration of the litter quality parameters between the species and cross types with ANOVA, followed by Tukey's *post hoc* tests to discern litter quality differences among tree types.

To compare the litter quality of the species and cross types in the common garden to field values, we also established nine 0.07 m² litter traps in each of 12 stands along the Weber River ($n = 108$ littertraps total). The field litterfall samples were collected on a monthly basis at each of the 12 stands (four each in the Fremont, hybrid and narrowleaf zones, with each stand separated by at least 1 km) throughout the Weber River drainage (41.2°N, 112.0°W) for 3 years (1998–2001). Here, we only report the results of the phenological year corresponding with the common garden values (i.e. 2000). The 12 stands were dominated by cottonwood of similar size and density, spanned 100 km with an elevational increase of 500 m. As a result of the morphological similarity of backcross hybrids and narrowleaf cottonwood in the field, those litter categories were pooled and compared with the average of the backcross hybrids and narrowleaf chemistry values from the common garden. No statistical analysis was made between the common garden and field chemical traits because chemical analyses were conducted on pooled samples within each stand.

Chemical analyses

We quantified the initial litter quality from ground subsamples of the pooled litter of each species and cross type from the common garden and the field. Material from both green leaf and litter was ground to a powder, exhaustively extracted for condensed tannins with 70% acetone + 10 mM ascorbic acid, and then assayed with the butanol–HCl method to determine condensed tannin concentrations (Porter *et al.* 1986). Standards used in these analyses were condensed tannin purified from narrowleaf cottonwood via the method of Hagerman & Butler (1989). We quantified lignin concentrations with the acetyl-bromide

method compared with an NIST certified pine standard (Iiyama & Wallis 1990). Litter total N and phosphorus (P) were determined by modified micro-Kjeldahl digestion (Parkinson & Allen 1975) and analysed on a Lachat AE Flow-Injection Auto-Analyzer (Lachat, Inc., Loveland, CO, USA), using the salicylate and molybdate-ascorbic acid methods, respectively. All chemical values are reported on an oven-dry (70 °C) mass basis.

Leaf litter decomposition

To separate the effects of litter quality from the effects of environment on decomposition, we placed leaf litter from the common gardens in mesh litterbags and left them to decompose in 10 field stands for 18 months. Litterbags were originally placed in all 12 stands, however vandalism in two of the stands (one stand each in the Fremont and hybrid zones) reduced our sample size to 10 stands. We placed 3–5 g of air-dry litter, of each species and cross type, in 12.5 cm × 12.5 cm mesh bags. The mesh size was 2 mm on top to allow colonization by soil mesofauna, and 0.5 mm on the bottom to minimize loss of material through fragmentation. We placed five replicates of each tree type in litterbags that were collected at four different dates each (four litter types × five replicates × four collection dates × 10 stands = 800 total litterbags). Each bag was secured to the forest floor with steel pins. The litterbags were placed on the ground in November, within 6 weeks of litter senescence to simulate the natural timing of litter deposition on the forest floor. Litterbags were placed randomly throughout each stand, in four clusters of 20, and were collected after 2, 6, 12 and 18 months in the field.

After removal from the field, the litterbags were air-dried for 2 weeks, all soil and other biotic contaminants were removed by hand and the samples were weighed. Decomposition rate constants were calculated as the negative slope from the linear regression of the natural logarithm of the fractional mass remaining at each collection date (Schlesinger & Hasey 1981). All mass values were expressed on an ash-free (500 °C for 1 h), oven-dry (70 °C for 48 h) mass basis. We compared litter quality parameters (i.e. litter total N, P, condensed tannin, lignin, condensed tannin : N, lignin : N ratios) to mean decay rates for each litter type ($n = 4$; *P. fremontii*, *P. angustifolia*, F₁ hybrid and backcross hybrid) with linear regression.

Nitrogen mineralization

In-field incubations for assessing soil net N mineralization rates (Raison *et al.* 1987; Hart *et al.* 1994a) were conducted from March 2000–2001, with four sequential incubations periods during the year. At each of eight sampling locations in the 12 field stands, we inserted two polyvinyl chloride

tubes (10.2 cm diameter × 20.3 cm in length) into the soil to a depth of 15 cm. The eight sampling locations were determined randomly at each site ($n = 16$ cores per stand per collection date). One of the paired tubes was removed immediately to determine initial soil inorganic-N pools. The other tube was covered with a cap (to prevent leaching) with 3-mm holes drilled on either side of the core (to allow gas exchange) and incubated *in situ*. Soils were incubated in each site over periods when soil moisture remained relatively constant in bulk soils (Raison *et al.* 1987). The spring, summer and fall incubations lasted 2.5 months each, while the winter incubation was 4.5 months in duration. A sub-sample of each soil (initial and incubated) was sieved field moist (4-mm sieve), extracted with 100 ml of 2 M KCl and analysed for inorganic N with a Lachat Flow-Injection Auto-Analyzer. Net N mineralization was calculated by subtracting the initial pool size of inorganic-N from the post-incubation pool size of inorganic-N. Annual rates of net N mineralization were determined by summing seasonal rates.

We conducted laboratory incubations with soil from each stand to separate the effects of the quality of soil organic matter as a substrate for net N mineralization (substrate quality) from environmental factors that influence the net N mineralization rates that we observed at each site under field conditions (Raison *et al.* 1987; Hart *et al.* 1994a,b). Eight soil cores (0–15 cm) were taken at random locations from all 12 sites. Soil samples were then sieved field moist (4-mm), thoroughly mixed and two 20-ml sub-samples were placed in 120-ml specimen containers and brought up to field capacity moisture content ($c. -33$ kPa matric potential). One of the sub-samples was immediately extracted with 100 ml of 2 M KCl and processed in the same manner as the field samples; these samples were used to estimate the initial inorganic-N pool size. The second sub-sample was incubated for 28 days in sealed, 0.944-l Mason jars placed in a dark growth chamber at 18 °C (mean soil temperature during the growing season averaged across all the sites). A quantity of 30 ml of deionized water was placed at the bottom of each jar to minimize soil water loss during the incubation and the jars were periodically flushed with ambient air to maintain aerobic conditions. After 28 days, the incubated soil samples were extracted with 100 ml of 2 M KCl and processed in the same manner as the field and pre-incubation initials (Raison *et al.* 1987; Hart *et al.* 1994a). Net N mineralization rates from both the laboratory and field incubations were expressed relative to soil total N to eliminate effects of site differences in soil N stocks on nutrient transformation rates (Powers 1990) and compared with ANOVA. Soil total N was determined with a Thermo-Quest (Stradavoltana, Italy) elemental carbon–nitrogen analyzer using a certified NIST soil standard from soils collected from the pre-incubation soil core of the last field incubation date. We used linear

regression to relate environmental parameters and litter quality inputs from each of the 12 field stands to net N mineralization rates. All statistical analyses were conducted with SPSS 8.0 with $\alpha \leq 0.05$ to assess statistical significance.

RESULTS

Along the Weber River in northern Utah, USA, condensed tannin concentrations differ widely between the cottonwood parental species and their hybrids. Mean concentration of condensed tannins in pooled leaf litter, from the common garden (Fig. 1a) and field (Fig. 1b), show that narrowleaf cottonwood genotypes have nearly 10 times more condensed tannins than Fremont genotypes. The F_1 generation

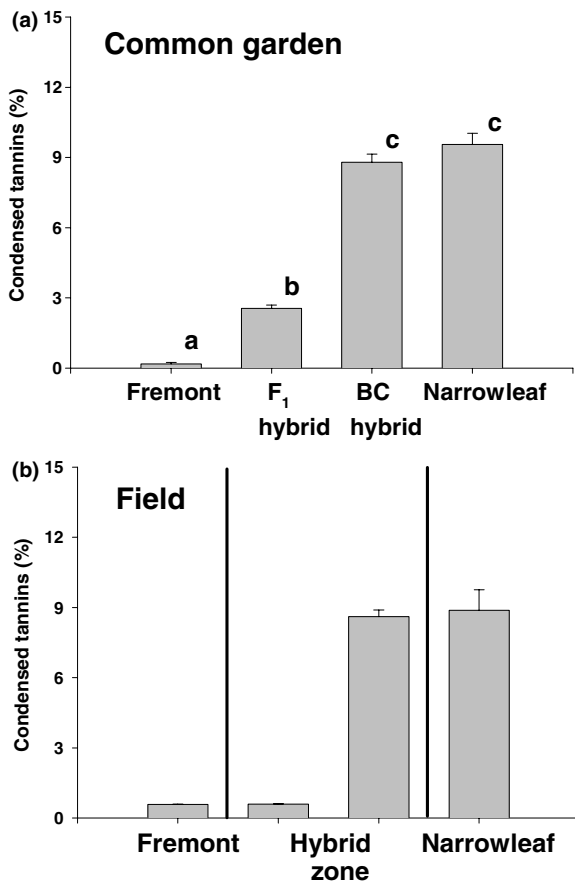


Figure 1 In a common garden, the species and cross types (genotypes pooled) express variation in an important trait that impacts leaf litter quality. The species and cross types vary 10 times in condensed tannin concentration (a), a pattern that is also found in the field (b). Because of difficulties in discriminating between backcross hybrids and narrowleaf cottonwood in the field, they have been presented together. Different lowercase letters between litter types indicate significant differences at the $\alpha = 0.05$ level. Vertical bars represent 1 SE of the mean.

expressed intermediate concentrations of condensed tannins, and the advanced backcross generations expressed some of the highest concentrations ($F = 5.47$, $P < 0.001$, d.f. = 3). We found that the species and cross types also range from 10.5–15.2% dry weight in lignin and 0.76–1.24 and 0.07–0.11% dry weight for N and P, respectively (data not shown).

The large qualitative differences in condensed tannin production between cross types occurring both under the same environmental conditions in a common garden and at multiple sites in the field suggest that condensed tannin concentrations were genetically based and heritable. Furthermore, the differences in condensed tannins are retained in the leaves after abscission, and concentrations are highly correlated between green leaves and leaf litter ($r^2 = 0.86$, $P < 0.0001$; data not shown). Because Fremont and narrowleaf cottonwood differ markedly in the concentration of condensed tannins, we predicted that there should be a strong negative relationship between the proportion of Fremont cottonwood alleles and foliar condensed tannins in individual genotypes. The confirmation of this prediction ($F = 31.30$, $r^2 = 0.63$, $P < 0.0001$, d.f. = 39; Fig. 2) demonstrates the segregation of alleles that are related to condensed tannin production.

The differential production of condensed tannins among these tree types is important because their concentration in leaf litter has a large effect on leaf litter decomposition. By placing all litter types (*P. fremontii*, *P. angustifolia*, F_1 hybrid

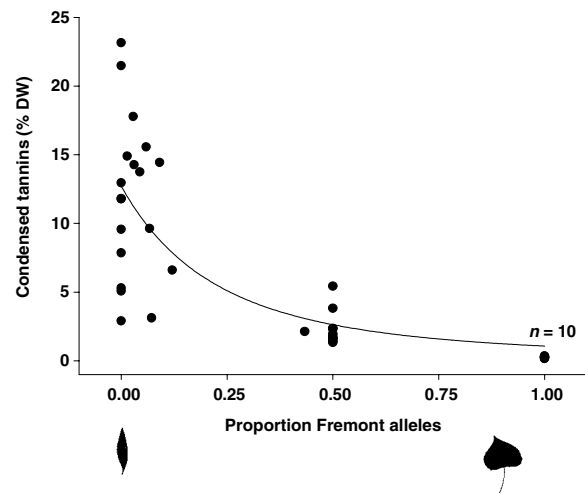


Figure 2 A significant negative (non-linear) relationship exists between proportion of Fremont marker alleles and green-leaf condensed tannin concentrations for 10 genotypes each of Fremont cottonwood, narrowleaf cottonwood and their F_1 and backcross hybrids ($F = 31.30$, $r^2 = 0.63$, $P < 0.0001$, d.f. = 39). This relationship indicates that alleles that control the regulation of this chemical trait are lost during introgression of Fremont genes into hybrid genotypes.

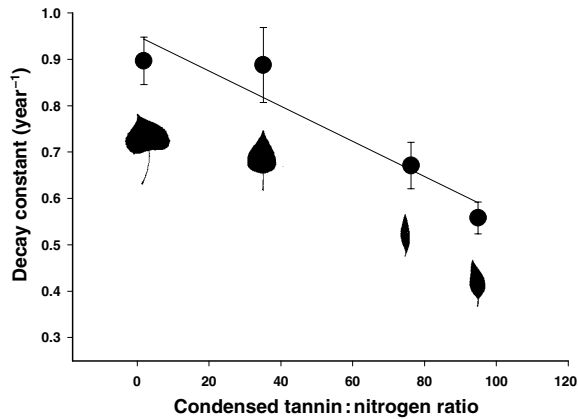


Figure 3 Litter decay rates of cottonwood litter types are significantly related to the initial ratio of foliar condensed tannins to total nitrogen that the tree types express. Only condensed tannin : N ratio was significantly related to decay ($F = 15.67$, $r^2 = 0.89$, $P = 0.05$, d.f. = 11).

and backcross hybrids of known genotype) collected from the common garden in 10 different gallery cottonwood forest sites (three in the Fremont zone, three in the hybrid or overlap zone and four in the narrowleaf zone), we were able to examine the effects of tree type, condensed tannin concentration and environment on rates of decomposition. We found that the ratio of the concentration of condensed tannins to total N in the initial litter was a better predictor of decomposition than any other measured litter quality trait ($F = 15.67$, $r^2 = 0.89$, $P = 0.05$, d.f. = 3; Fig. 3). Total N ($F = 0.04$, $r^2 = 0.02$, $P = 0.86$, d.f. = 3), total P ($F = 0.34$, $r^2 = 0.15$, $P = 0.62$, d.f. = 3), total condensed tannins ($F = 6.49$, $r^2 = 0.76$, $P = 0.13$, d.f. = 3), total lignin ($F = 8.75$, $r^2 = 0.81$, $P = 0.10$, d.f. = 3) and lignin : N ($F = 1.17$, $r^2 = 0.37$, $P = 0.39$, d.f. = 3) were not significantly related to decay. This effect on decomposition is consistent with other findings where condensed tannins in litter influenced ecosystem-level processes by inhibiting saprotrophic fungi and soil microfauna, and by forming protein–tannin complexes that act to retain N in ecosystems (Horner *et al.* 1988; Northup *et al.* 1995; Schimel *et al.* 1995; Northup *et al.* 1998).

Similarly, across 12 stands of gallery cottonwood forest, we found that the amount of condensed tannin in litterfall was the best predictor of soil net N mineralization (conversion of organic-N to plant-available inorganic-N). Under both field and laboratory conditions, the composition of trees at each site and the amount of condensed tannin they expressed accounted for a large proportion of the variation in net N mineralization. Under field conditions, net N mineralization differed significantly among the four field sites in the Fremont, hybrid and narrowleaf zones

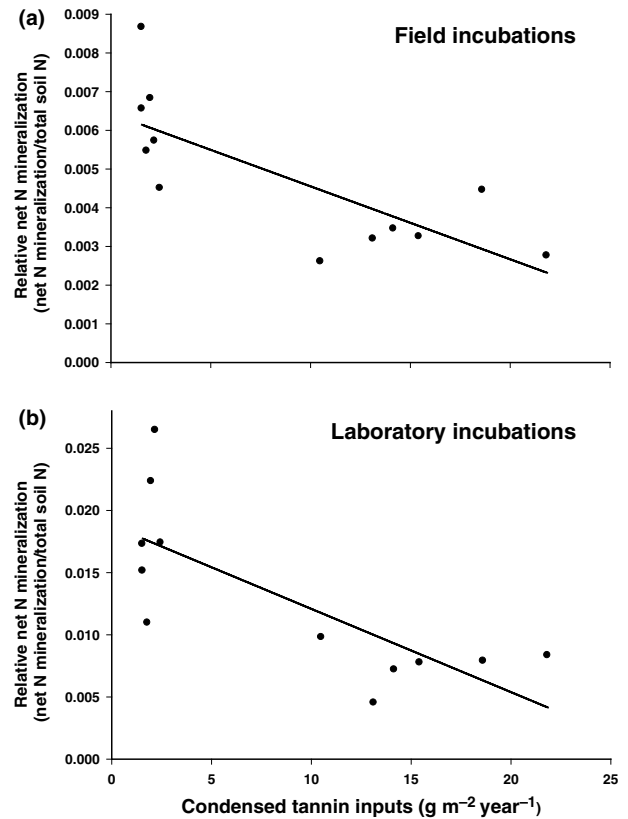


Figure 4 In both field and laboratory incubations, condensed tannin in litterfall was best related to soil net N mineralization. Condensed tannins in litterfall explained 65% of the variation in annual rates of net N mineralization in soils from 12 stands of gallery cottonwood forests of varying composition in the field (a; $F = 16.45$, $r^2 = 0.65$, $P = 0.003$, d.f. = 11). This variable also explained 55% of the variation in net N mineralization over a 28-day laboratory incubation where moisture and temperature were the same across all soils (b; $F = 11.09$, $r^2 = 0.55$, $P = 0.009$, d.f. = 11). In both cases, rates of net N mineralization are expressed relative to the soil total N concentration in order to account for differences in the total amount of soil N found among sites (Powers 1990).

($F = 4.04$, $P = 0.02$, d.f. = 2; data not shown) and indicate that overall, these sites are relatively nutrient poor, relative to other forest sites (Scott & Binkley 1997). On an individual site basis, condensed tannin amounts in litterfall explained 65% of the variation in net N mineralization rate ($F = 16.45$, $r^2 = 0.65$, $P = 0.003$, d.f. = 11; Fig. 4a). In laboratory incubations of these same soils where environmental conditions (i.e. temperature and moisture) were held constant across sites, condensed tannins still best predicted net N mineralization, explaining 55% of the variation across sites ($F = 11.09$, $r^2 = 0.55$, $P = 0.009$, d.f. = 11; Fig. 4b). In contrast to other field observations (Scott & Binkley 1997), lignin ($F = 2.63$, $r^2 = 0.23$, $P = 0.14$, d.f. = 11) or

stand climatic variables such as mean annual soil temperature ($F = 0.43$, $r^2 = 0.05$, $P = 0.53$, d.f. = 11) or moisture ($F = 0.19$, $r^2 = 0.02$, $P = 0.68$, d.f. = 11) were not significantly related to net N mineralization rates. While these relationships do not demonstrate causation, they do suggest that variation in plant polyphenols is the dominant factor regulating N cycling in this ecosystem.

DISCUSSION

Our results demonstrate that genetic variation in a heritable plant trait affects ecosystem-level processes such as litter decomposition and soil net N mineralization. Such relatively direct effects argue that the impacts of this genetically based trait were strong relative to environmental influences. These data support recent reports suggesting that condensed tannins and other polyphenols in leaf litter from nutrient poor sites can influence the pools and fluxes of soil nutrients (Northup *et al.* 1995; Schimel *et al.* 1995; Northup *et al.* 1998; Hättenschwiler & Vitousek 2000), and support the argument that the expression of this trait can be important in nutrient poor sites (Northup *et al.* 1998; Hättenschwiler *et al.* 2003). Plant polyphenols, as a class of defensive or secondary compounds, are involved in plant pigmentation, ultraviolet protection, allelopathy and defense against herbivores and pathogens (Zucker 1982; Palo 1984; Hemingway & Karchesy 1989; Ossipov *et al.* 2001). They have been shown to retard leaf litter decomposition by reducing palatability to arthropods and microbes and by forming protein-binding complexes (Gallardo & Merino 1992; Grime *et al.* 1996; Heal *et al.* 1997; Driebe & Whitham 2000). The influence of polyphenols on decomposition processes and their ability to inhibit net nitrification have led researchers to hypothesize that these compounds can regulate nutrient pools available to plants (Basaraba & Starkey 1966; Rice & Panchoy 1973; Northup *et al.* 1998; Hättenschwiler & Vitousek 2000; Hättenschwiler *et al.* 2003).

This study contributes to the growing literature suggesting that genetic variation is linked to both communities (Duney *et al.* 2000) and ecosystem-level processes such as nutrient cycling, and therefore argues for the inclusion of genetic variation in ecosystem models (Whitham *et al.* 2003). Genetic variation in plants is a factor that could have large and predictable effects on ecosystem processes. Studies examining the effects of biodiversity on ecosystem function commonly focus only on variation among species, although intraspecific genetic variation could have equally important influences (Hooper *et al.* 2000; Knops *et al.* 2002). Interspecific studies have found idiosyncratic relationships between species diversity and ecosystem processes, such as productivity and nutrient cycling (Tilman *et al.* 1997; Loreau *et al.* 2001; Naeem 2001). Our findings argue that it is important

to quantify the genetic basis for ecologically important traits such as tannins that have extended phenotypes on ecosystem function. This goal represents a major frontier in ecosystem studies, as plant genes could provide the underlying framework for understanding many important interactions with associated communities (e.g. herbivore and/or microbial), which in turn are likely to affect ecosystem function. The effect of genetic variation on nutrient cycling could be substantial because plant genes have the potential to impact the amount and chemical quality of plant litter added to ecosystems (Treseder & Vitousek 2001; Lindroth *et al.* 2002; Madritch & Hunter 2002).

These findings indicate the utility of a genetic approach to understand complex systems and to unify diverse fields from genetics to ecosystem studies. Perhaps, most importantly, a genetic approach has the potential to explain ecosystem function within an evolutionary framework. Elucidating the extended effects of plant genes at the ecosystem level demonstrates a previously unconfirmed heritable component to large scale processes and suggests that the establishment of these connections can affect the accuracy and predictability of models of ecosystem function. Lastly, a link between specific plant genes and ecosystem processes indicates that intraspecific genetic variation may affect both species survival and ecosystem function, which has major conservation implications.

ACKNOWLEDGEMENTS

We thank C. Grow, M. Dahnert, G. Cox, J. Andrade, S. Chapman, G. Wimp, R. Bangert, D. Fischer, L. Wiczorek and S. Woolbright for assistance in the field and lab. We thank the Whitham and Hart lab groups, and D.A. Wardle and three anonymous reviewers for their thoughtful comments on the manuscript. This research was supported by NSF grants DEB-0078280, DEB-0087017, DEB-0075563, DEB-9726504, DEB-0074427, DEB-9306981 and USDA NRICGP grant 95-37302-1810.

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Editor, D. Wardle

Manuscript received 9 September 2003

First decision made 19 October 2003

Manuscript accepted 28 November 2003