

MINIREVIEW

From Genes to Ecosystems: The Genetic Basis of Condensed Tannins and Their Role in Nutrient Regulation in a *Populus* Model System

Jennifer A. Schweitzer,^{1*} Michael D. Madritch,² Joseph K. Bailey,¹
Carri J. LeRoy,³ Dylan G. Fischer,³ Brian J. Rehill,⁴
Richard L. Lindroth,² Ann E. Hagerman,⁵ Stuart C. Wooley,⁶
Stephen C. Hart,⁷ and Thomas G. Whitham⁸

¹Department of Ecology and Evolutionary Biology, University of Tennessee, 569 Dabney Hall, Knoxville, Tennessee 37996, USA; ²Department of Entomology, University of Wisconsin, Madison, Wisconsin, USA; ³Evergreen State College, Olympia, Washington, USA; ⁴Department of Chemistry, U.S. Naval Academy, Annapolis, Maryland, USA; ⁵Department of Chemistry and Biochemistry, Miami University, Oxford, Ohio, USA; ⁶Department of Biological Sciences, California State University, Stanislaus, Turlock, California, USA; ⁷School of Forestry and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, Arizona, USA; ⁸Department of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, Arizona, USA

ABSTRACT

Research that connects ecosystem processes to genetic mechanisms has recently gained significant ground, yet actual studies that span the levels of organization from genes to ecosystems are extraordinarily rare. Utilizing foundation species from the genus *Populus*, in which the role of condensed tannins (CT) has been investigated aboveground, belowground, and in adjacent streams, we examine the diverse mechanisms for the expression of CT and the ecological consequences of CT for forests and streams. The wealth of data from this genus highlights the importance of form and function of CT in large-scale and long-term ecosystem processes and demonstrates the following four patterns: (1) plant-

specific concentration of CT varies as much as four-fold among species and individual genotypes; (2) large within-plant variation in CT occurs due to ontogenetic stages (that is, juvenile and mature), tissue types (that is, leaves versus twigs) and phenotypic plasticity in response to the environment; (3) CT have little consistent effect on plant–herbivore interactions, excepting organisms utilizing woody tissues (that is, fungal endophytes and beaver), however; (4) CT in plants consistently slow rates of leaf litter decomposition (aquatic and terrestrial), alter the composition of heterotrophic soil communities (and some aquatic communities) and reduce nutrient availability in terrestrial ecosystems. Taken together, these data suggest that CT may play an underappreciated adaptive role in regulating nutrient dynamics in ecosystems. These results also demonstrate that a holistic perspective from genes-to-ecosystems is a powerful approach for elucidating

Received 24 January 2008; accepted 1 July 2008; published online 21 August 2008

All authors made significant contributions of data, research or writing to the study described in this review.

*Corresponding author; e-mail: Jen.Schweitzer@utk.edu

complex ecological interactions and their evolutionary implications.

INTRODUCTION

Since Feeny (1968, 1969, 1970) researchers have consistently sought to understand the adaptive significance of condensed tannins (CT) in plants. Much of this work has investigated the role that CT play in plant resistance to herbivores (Rhodes and Cates 1976; Swain 1979; Bernays and others 1989). A parallel body of research, however, has focused instead on the role of CT in regulating soil nutrient availability suggesting another, but less widely accepted, adaptive role for CT (Basaraba and Starkey 1966; Northrup and others 1998; Hättenschwiler and Vitousek 2000; Kraus and others 2003). The ability of CT to regulate the soil nutrient environment implicitly links above- and belowground processes, as soil nutrient availability establishes the foundations for plant fitness and performance, and plant nutrient status often affects herbivore fitness and performance. Utilizing the breadth of studies on the expression and ecological consequences of CT in a single genus (*Populus* spp.), from individuals to ecosystems, we examine the potential adaptive role of CT as an important linkage between above- and belowground processes as well as between forests and streams.

Condensed tannins (proanthocyanidins) are complex flavonoid polymers that influence many ecological processes in both terrestrial and aquatic ecosystems. In most Northern hemisphere plants, condensed tannins are composed of the four flavan-3-ols catechin, epicatechin, galocatechin and epigallocatechin. Like hydrolyzable tannins, condensed tannins are potent protein-binding agents, highly redox reactive, and good ligands for many metal ions. However, the two classes of tannins are distinct in their biosynthetic origins, their subunit composition, and their mode of polymerization (Hagerman 1992). In many plant species, CT have been shown to affect herbivore resistance (Feeny 1970; Cates and Rhodes 1977; Bernays and others 1989; Forkner and others 2004; Kosola and others 2006), influence microorganisms and their activities (Scalbert 1991; Field and Lettinga 1992; Athanasiadou and Kyriazakis 2004; Iason and Vilalba 2006; Madritch and others 2007a, b) and alter mycorrhizal interactions (Bending and Read 1996; Northrup and others 1998; Bradley and others 2000; Kosola and others 2006). In turn these effects

Key words: above- and belowground interactions; aquatic–terrestrial linkages; condensed tannin; community genetics; *Populus*; plant–soil feedbacks; Salicaceae.

(especially on microorganisms) may slow rates of leaf litter decay (Basaraba and Starkey 1966; Benoit and Starkey 1968; Horner and others 1988; Stout 1989; Ostrofsky 1997), and nitrogen (N) mineralization (Schimel and others 1996; Northrup and others 1998; Verkaik and others 2006; Nierop and others 2006) thus influencing overall N availability within ecosystems (Gallardo and Marino 1992; Schimel and others 1998; Hättenschwiler and Vitousek 2000; Kraus and others 2004). A rich literature indicates that CT generally inhibit organisms, from microbes to mammals, although this effect often depends on the structure of the compound and the ecological context (Ayres and others 1997; Madritch and others 2007b). High-molecular weights and astringent properties of CT have been shown to reduce the palatability of plant tissues with high CT to both vertebrate and arthropod herbivores as well as reduce the nutritive value of plant tissues by binding proteins and other molecules into indigestible tannin complexes in the gut (Robbins and others 1987; Bernays and others 1989; Fahey and Jung 1989; Hagerman and others 1992; Bhat and others 1998). Similar effects of CT have been found in the soil: tannin-protein complexes may slow nutrient availability or even make mineral nutrients unavailable to plants, except by the actions of some mycorrhizal species (Northrup and others 1998 and references therein). Soil microbes are often inhibited by CT of large molecular weight but can also use small molecular weight CT as a carbon (C) source (more below). These inhibitory and nutrient binding properties as well as other possible roles of CT, such as protection of leaves from photo-oxidation (Close and McArthur 2002), argue several possible adaptive roles for the production of this polymer in plants.

Condensed tannins occur across phylogenetically diverse plant groups in ecosystems from arctic to tropical latitudes, suggesting a deep evolutionary history. Common garden studies (in the absence of environmental variation) have shown that production of CT is genetically mediated at both large and small taxonomic scales. For instance, CT are common in woody plants, but almost absent in herbaceous species (Haslam 1988). For CT to be evolutionarily important, in most cases (that is, except where traits are fixed), there must also be

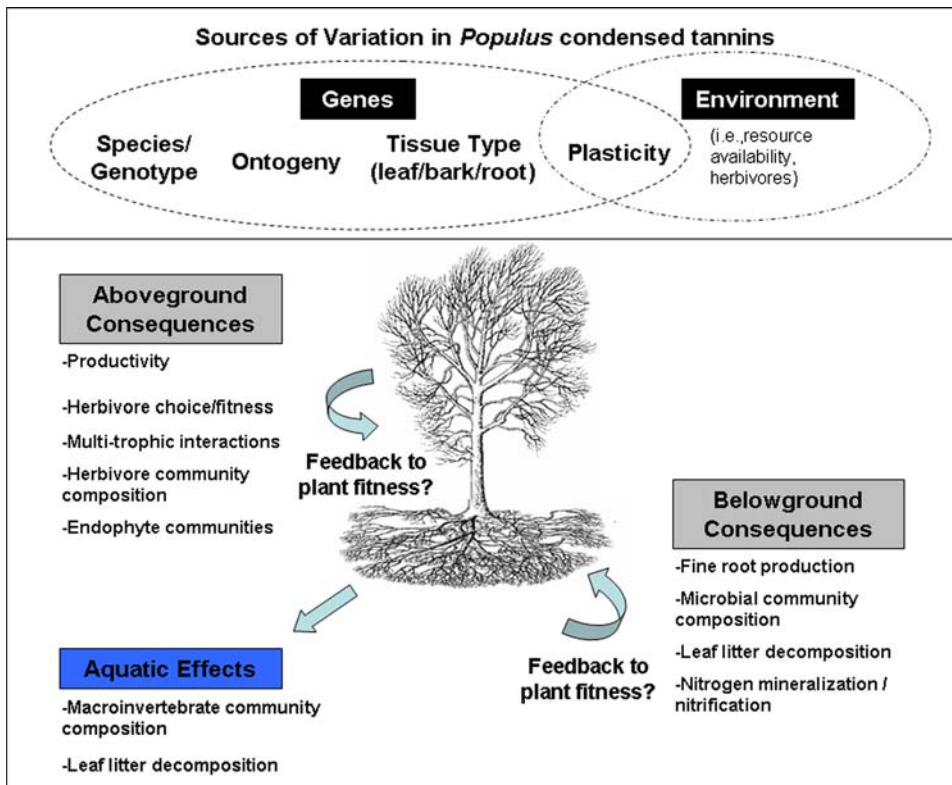


Figure 1. Conceptual model illustrating the interacting effects of both plant genes and the environment (for example, resource availability and herbivores) on variation in production of condensed tannins (CT) in *Populus*, as well as the range of aboveground, belowground, and aquatic effects due to this variation. Utilizing the wealth of data available from *Populus*, we examine the relative strength of factors that influence the expression of CT and the above- and belowground consequences of this variation to understand the selective pressures on CT in *Populus* ecosystems.

significant intraspecific genetic variation and heritability of this trait. For example, in the genus *Leucaena* grown in a common garden environment, Dalzell and Shelton (2002) found an approximately 145-fold difference in concentration among individual genotypes with the production of CT ranging from 0 to 336 g CT/kg litter, demonstrating high intraspecific variation. Likewise, genetically mediated variation in the concentration of foliar CT between replicated genotypes grown in a common environment has been demonstrated in several genera including *Quercus*, *Eucalyptus*, *Meterosideros*, and *Populus* (for example, Dungey and others 2000; Treseder and Vitousek 2001; Madritch and Hunter 2002; O'Reilly-Wapstra and others 2005; Rehill and others 2006; Osier and Lindroth 2006). Variation in the strength and direction of the influence of CT on ecological processes indicates that the effects of CT strongly depend upon plant species, ecological context, tissue type and/or the specific molecular configuration of the polymer (Fierer and others 2001; Kraus and others 2003; Nierop and others 2006). Although many recent advances have increased our understanding of the genomic and molecular machinery of the expression of CT in plants (Marles and others 2003; Dixon and others 2005), here, we examine the ecological and possible evolutionary consequences of variation in the production of this secondary compound.

Figure 1 outlines a conceptual model of the interacting factors that influence CT in *Populus*. It illustrates all of the components known to impact the production of CT as well as how this variation in production then affects associated populations and communities of organisms as well as ecosystem function. Focusing on this model of CT in *Populus* and the growing literature on this single genus—from molecular genetics to ecosystem ecology—we examine how this genetically controlled variation interacts with spatially and temporally variable selective forces to shape the evolution of CT in *Populus*, as well as traits of the communities and ecosystems in which they are imbedded as foundation species. Specifically, we use this model to examine: (1) the factors known to influence CT and (2) the relative importance of CT on aboveground, belowground, and aquatic processes, concluding with an examination of the possible fitness consequences of these variable interactions. In combination, analyses of these factors further our understanding of the causes as well as the ecological and evolutionary consequences of CT in *Populus*.

Populus as a Model System

The genus *Populus* is a model foundation forest tree to examine the ecological and evolutionary con-

Table 1. Reported Effects of Condensed Tannin Concentrations across Levels of Organization in *Populus*

Effect	Scale	Direction (R^2) ¹	Tissue	References
Population				
Gypsy moth/tent cat. Performance	Clone ²	Variable/NS	Leaf	Hwang and Lindroth (1997)
Tiger swallowtail/sphinx moth performance	Clone ²	NS	Leaf	Hwang and Lindroth (1998)
Parasitoid performance	Clone ²	Negative (0.33)	Leaf	Holton and others (2003)
Beaver choice	Hybrid Complex ³	Negative (0.46)	Twig	Bailey and others (2004)
Beetle larval growth	Clone ²	Negative (0.21)	Leaf	Donaldson and Lindroth (2004)
Aphid gall density	Hybrid Complex ³	Positive (0.43)	Leaf	Bailey and others (2006)
Elk choice	Clone ²	NS	Leaf	Wooley and others (2008)
Community				
Endophyte infection	Hybrid Complex ³	Negative (0.54)	Twig	Bailey and others (2005)
Gall–bird interaction	Hybrid Complex ³	Positive (0.39)	Leaf	Bailey and others (2006)
Arthropod	Hybrid Complex ³	Positive/Negative	Leaf	Whitham and others (2006)
Stream invertebrates	Hybrid Complex ³	Positive/Negative	Leaf Litter	Whitham and others (2006)
Microorganisms	Genotype ⁴	Positive/Negative	Soil/Litter	Schweitzer and others (2007)
Ecosystem				
N ² fixation	Species ⁵	Negative	CT extracts	Cates and Rhodes (1977)
Litter decomposition (terr)/nutrient immobilization	Species ⁵	Negative	CT extracts	Schimel and others (1996)
Litter decomposition (aq)	Hybrid Complex ³	Negative (0.63)	Leaf litter	Driebe and Whitham (2000)
Litter decomposition (terr)	Hybrid Complex ³	Negative (0.89)	Leaf litter	Schweitzer and others (2004)
Net N mineralization	Species ⁵	Positive/Negative	CT extracts	Fierer and others (2001)
Net N mineralization	Hybrid Complex ³	Negative (0.65)	Litter inputs	Schweitzer and others (2004)
Litter decomposition (aq)	Hybrid Complex ³	Negative (0.97)	Leaf litter	LeRoy and others (2006)
Root production	Hybrid Complex ³	Positive (0.60)	Root	Fischer and others (2006)
Litter decomposition (terr)	Genotype ²	Positive/Negative	Leaf litter	Madritch and others (2006)
Litter decomposition (aq)	Hybrid complex ³	Negative (0.72)	Leaf litter	LeRoy and others (2007)

(Terr) and (Aq) represent studies completed in terrestrial and aquatic habitats, respectively.

¹If no R^2 value is presented, it was not quantified in the study.

²*P. tremuloides*.

³*P. fremontii* × *P. angustifolia*.

⁴*P. angustifolia*/backcross hybrid.

⁵*P. balsamifera*.

sequences of CT, as *Populus* forests are a dominant component of several terrestrial landscapes and CT are generally abundant in the genus. Condensed tannin can comprise up to 35% dry weight of a *Populus* leaf and demonstrate substantial quantitative variation among genotypes (Lindroth and Hwang 1996; Bailey and others 2006; Madritch and others 2006; Rehill and others 2006; Tsai and others 2006). Consequently, recent work elucidates the role of the quantity (that is, concentration), quality (that is, molecular structure) and genetic determinants of CT as well as the ecological effects of the expression of CT at the population, community and ecosystem levels (Table 1; Whitham and others 2006, 2008).

Although excellent research has been conducted in other plant systems concerning the effects of CT on associated organisms and processes, only in *Populus* has extensive genetic and ecological re-

search (including both terrestrial and aquatic studies) addressed CT within both natural field populations and experimental frameworks. *Populus* includes the first woody species (*Populus trichocarpa*) to have its entire genome sequenced and recent work indicates a strong genetic component to the accumulation of CT in plant tissues (Harding and others 2005; Tuskan and others 2006; Ralph and others 2006; Tsai and others 2006). Using quantitative genetic approaches, CT in *Populus* spp. have demonstrated clonal repeatability (that is, broad-sense heritability; Conner and Hartl 2004; Stevens and Lindroth 2005; Bailey and others 2006; Donaldson and Lindroth 2007; Lindroth and others 2007). For example, replicate plants grown in common environments have demonstrated that genetic factors can explain up to 52% of the variation in production of CT in *P. angustifolia* genotypes (Bailey and others 2006). Using a population

of *P. fremontii* and *P. angustifolia* crosses, the expression of CT has been mapped using Quantitative Trait Loci (QTL) methodologies (Woolbright 2001; Whitham and others 2006). Two putative QTL have been identified, which correspond with two specific regions of the genome and production of CT. Most recently, research by Tsai and others (2006), using 100 annotated *Populus* shikimate-phenylpropanoid pathway genes (and expression data for half of these) identified specific genes or gene families correlated with CT under both constitutive and induced conditions. They noted that *Populus* gene families associated with production of flavonoids and the downstream CT are quite large, consistent with the substantial quantitative commitment to phenolic biosynthesis in the genus. Therefore, although no individual genes have been identified to date, both quantitative and molecular genetic approaches indicate specific genetic mechanisms for variation in CT in general. Such strong evidence for genetic control of variation in CT suggests a significant evolutionary role.

PATTERNS OF CONDENSED TANNIN PRODUCTION IN *POPULUS*

Figure 1 illustrates the role of plant genes interacting with the environment to predictably influence the accumulation of CT between species, between ontogenetic stages, between tissue types and how the environment (both biotic and abiotic) can influence plasticity in these patterns. Here we outline the known patterns of accumulation of CT in *Populus* to understand its potential adaptive significance.

Qualitative Variation Between Species/Genotypes

Recent and ongoing studies of CT in *Populus* indicate that the specific effects of CT on ecological processes, in general, may be the result not only of quantitative but qualitative variation in the specific structure and stereochemistry of the tannin polymer. A growing literature shows that the molecular weight and configuration of CT are species- (and potentially genotype-) specific, and have important effects on the ecological consequences of CT (*sensu* Clausen and others 1990; Fierer and others 2001; Kraus and others 2003; Nierop and others 2006; A. Hagerman unpublished data). For example, the size and structure of the polymer can be directly related to inhibitory effects on herbivores or microorganisms. Across both interspecific and intraspecific levels of examination, variation in the molecular

weight and configuration of CT in *Populus* is just beginning to be examined and the data are revealing large differences in the number and type of monomer extender units (Figure 2). Preliminary data suggest that *P. tremuloides* and *P. fremontii* both have a small number of extender units (8–11 and 0–5, respectively). However, the extender units of *P. tremuloides* polymers are composed of epicatechin, galocatechin, and epigallocatechin, whereas those of *P. fremontii* are primarily epicatechin. In contrast, the CT of *P. angustifolia* and backcross hybrids are comprised of long chain lengths (15–20) of epicatechin and epigallocatechin units (A. Hagerman unpublished data). Little is understood about the relative ecological influence of this variation in extender complexity. CT comprised of ortho-trihydroxyl substituted flavan-3-ols (galocatechin and epigallocatechin subunits) oxidize more rapidly than CT comprised of dihydroxylated subunits (catechin and epicatechin), which may have significance for both biotic and abiotic reactions (Barbehenn and others 2006). Extender complexity may be important to both herbivores and microbial heterotrophs as qualitative variation in CT can influence whether CT are inhibitory or toxic to these organisms or, in the case of microorganisms, if CT are used as an energy source (Madritch and others 2007b). Hence, the molecular structure of the compound may ultimately impact many aspects of both above- and belowground species interactions (Scalbert 1991; Kraus and others 2003; Fierer and others 2001; Bradley and others 2000; Kanerva and others 2006; Nierop and others 2006).

Introgression

Overall relationships between CT and various ecological processes tend to be stronger across an introgression gradient (*P. fremontii* × *P. angustifolia*) than within a species. For example, studies across a hybridizing complex (correlating CT with diverse response variables) show that the concentration of CT can explain between 39 and 97% of the variation in the ecological trait of interest (for example, herbivore resistance, community response, litter decay rate, and so on). However within a species, variation in concentration of CT between genotypes can explain (in studies to date) between 16 and 28% of the variation in ecological traits (Table 1).

Genetic introgression across a *P. fremontii* × *P. angustifolia* hybridizing complex can alter the chemistry of CT via both additive and epistatic expression of genes for CT. The introgression of

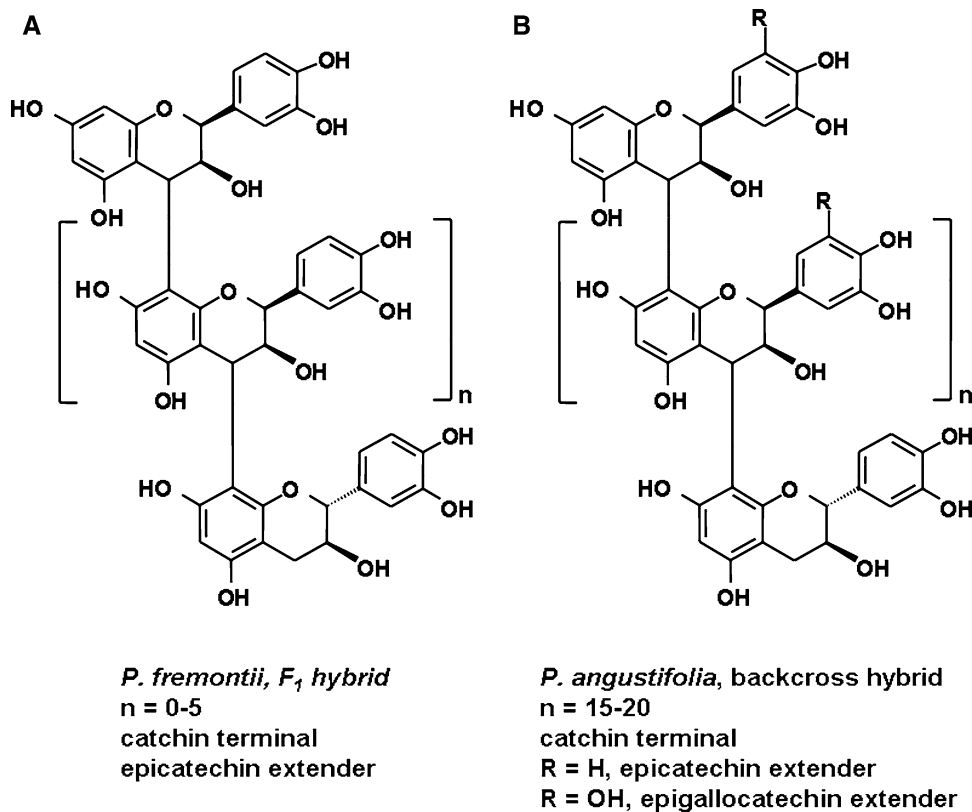


Figure 2. Qualitative differences in condensed tannins between species and crosstypes in natural *Populus fremontii* × *P. angustifolia* crosses. All the tannins have catechin terminal units, but *P. fremontii* and F_1 hybrids have short chains comprising 0–5 epicatechin extender units (A), whereas *P. angustifolia* and backcross hybrids have longer chains made up of 15–20 epicatechin and epigallocatechin extender units (B).

P. fremontii marker alleles is negatively related to the concentration of CT in foliar and woody tissues, explaining between 78 and 93% of the variation across multiple studies and years (Bailey and others 2002, 2004; Schweitzer and others 2004; LeRoy and others 2006; Fischer and others 2006). Trees with more *P. fremontii* markers have (on average) lower concentrations of CT in tissues, F_1 hybrids are intermediate and backcross hybrids show the highest variation and can even express non-additive (that is, dominant or epistatic) concentrations of CT. Moreover, CT across the hybridizing complex also vary qualitatively, with *P. angustifolia* and backcross genotypes having CT with two to three times the number of chain lengths that are half epicatechin and half epigallocatechin (described above). These data suggest the segregation of alleles with hybridization can impact both quantitative and qualitative variation in CT.

Ontogeny

Plant ontogeny (that is, a heritable change in gene expression as a function of plant age or canopy height; Lawrence and others 2003) can have substantial effects on leaf (and possibly root) chemistry within *Populus*. For example, ontogenetic shifts in tissue chemistry commonly occur in some *Populus*

species suggesting that phytochemical trajectory as a function of tree age or canopy height may have ecological consequences for herbivores and associated communities, as well as ecosystem processes (Waltz and Whitham 1997; Kearsley and Whitham 1998; Donaldson and others 2006; Rehill and others 2006; Lindroth and others 2007). *Populus fremontii* genotypes show little change in the concentration of CT from juvenile to adult foliage, whereas *P. angustifolia* show large variation (hybrids are intermediate; Rehill and others 2006). The concentrations of CT in *P. angustifolia* foliage increase with tree height, such that the mature (upper) portion of the canopy can have threefold higher concentrations of CT than leaves in the lower canopy that express juvenile traits. *Populus tremuloides* genotypes also show large ontogenetic shifts, switching from the production of phenolic glycosides to the production of CT with age, ultimately doubling leaf concentrations of CT within the first 5 years of growth (Donaldson and others 2006). Similarly, ontogenetic rejuvenation following coppicing of *P. tremuloides* saplings and re-growth of juvenile foliage from root sprouts showed relative increases in the concentrations of foliar CT from 5 to 72% (Lindroth and others 2007). The specific genetic mechanisms for the expression of CT and up-regulation in these species (across- and within-

species) is just beginning to be appreciated and functional genomic data suggest a complex balance of signaling networks and photosynthetic stimulation that differentially balances up-regulation (Xie and others 2003; Dixon and others 2005; Harding and others 2005; Tsai and others 2006). In contrast to the increasing concentration of CT in mature foliage relative to juvenile foliage, phenolic glycosides tend to decline with ontogenetic shifts to mature foliage in multiple *Populus* species (Rehill and others 2006; Donaldson and others 2006). The phenolic glycosides (prevailing in young trees) are more effective at defending against canopy herbivores than are CT (prevailing in older trees; Hemming and Lindroth 1995). This ontogenetic shift may indicate a tradeoff between herbivore defense at earlier life stages and regulation of the soil nutrient environment as the plant matures.

Tissue Type

Variation in the concentration and predictability of the expression of CT also occurs among tissue types (that is, leaves, twigs or roots). Studies in *P. tremuloides* have found clonal repeatability (H^2 or broadsense heritability; Conner and Hartl 2004) of CT in twig tissue to be much lower than in leaf tissues; twig tissue $H^2 = 0.38$ (Lindroth and others 2007) versus H^2 greater than 0.70 in foliage (Stevens and Lindroth 2005; Donaldson and others 2007). Variation exists between tissues in *P. fremontii* and *P. angustifolia* (and their natural hybrids) as leaves can have up to 5 times more CT than twig tissue and allocation of CT to roots is even smaller (Bailey and others 2005; Rehill and others 2006, D. Fischer unpublished data). Across species, results suggest that CT in stems, roots and other woody tissue may not be as plastic as leaves to environmental variation (Kosola and others 2006; Lindroth and others 2007). Little is known about qualitative variation in CT between plant tissues.

Phenotypic Plasticity

Quantitative variation in CT across *Populus* species is plastic in response to many variables, including changing global climates and environmental conditions (Mansfield and others 1999; Kausurina and others 2005; Kosola and others 2006; Osier and Lindroth 2006; Madritch and others 2006). A rich literature has documented quantitative differences in concentrations of CT with exposure to elevated CO_2 or O_3 , soil nutrient availability, and herbivory indicating high plasticity in the phenylpropanoid pathway (Lindroth and others 1993, 1997; Liu and

others 2005; Findlay and others 1996; Mansfield and others 1999). For example, exposure to elevated CO_2 (and/or N limitation) has been found to shift carbon allocation patterns such that the concentration of CT can increase up to 5 times, relative to controls, across genotypes of *P. tremuloides* (Mansfield and others 1999). Moreover these patterns in shifting allocation can be genotype and resource specific (Harding and others 2005; Madritch and others 2006). Osier and Lindroth (2006) found that only in high light conditions did genotypes decrease their production of CT with fertilization; whereas under low light conditions genotypes showed little plasticity in response to soil fertilization. Resource availability has also been shown to impact inducible chemical responses to herbivory (Lindroth and Hwang 1996; Osier and Lindroth 2001, 2004).

Recent research has identified specific regions of the genome that are expressed under different environmental regimes (that is, nitrogen limitation) indicating specific genetic mechanisms behind the plasticity in this trait (Harding and others 2005). Such research provides a molecular explanation to the carbon/nutrient allocation theory that predicts carbon-based secondary metabolites will increase under nutrient stress (Bryant and others 1983). Studies at the genotype level suggest that the phenylpropanoid pathways in foliar tissue of *Populus* is very plastic, and recent data suggests that plasticity itself is genetically controlled (Peters and Constabel 2002; Ralph and others 2006; Tsai and others 2006). Conversely, there is little evidence, so far, for phenotypic plasticity in qualitative (that is, structural) variation in CT with environmental variation. *P. angustifolia* genotypes grown in three common gardens, across a 500 m elevational gradient, demonstrated little variation in molecular weight or number and type of extender units (A. Hagerman, unpublished data) of the polymer.

ECOLOGICAL CONSEQUENCES OF CONDENSED TANNINS

The wealth of data to date within *Populus* suggest a genetic basis to the expression of CT between species, genotypes, tissue types, developmental stage and potentially phenotypic plasticity in response to environmental variation. These data also show that although the environment can have strong effects on quantitative (that is, concentration) variation in CT, environmental factors have little influence on qualitative variation (that is, polymer structure). Figure 1 illustrates the known ecological consequences of variation of CT in *Populus* for above- and

belowground processes and in streams. Here we examine the potential consequences of CT as a defensive compound, as a regulator of ecosystem function, or both.

Aboveground Effects

A rich literature from the past 40 years outlines potential defensive roles for CT against consumers, however, this defensive relationship is often context specific (Rhodes and Cates 1976; Swain 1979; Bernays and others 1989; Butler 1989; Schultz 1989; Forkner and others 2004). The relationship between foliar CT and aboveground herbivory in *Populus* is mixed, consistent with a broader literature in other plant species (Table 1; Ayres and others 1997; Iason and Vilalba 2006). For example, Bailey and others (2006) found positive correlations of both gall-forming aphid abundance and bird predation with foliar concentration of CT in *P. angustifolia* genotypes, whereas Rehill and others (unpublished data) have found negative correlations with larval beetle growth rate ($R^2 = 0.50$, $P < 0.001$). In contrast, *P. tremuloides* CT are negatively correlated with larval beetle growth rate ($R^2 = 0.21$, $P = 0.011$, Donaldson and Lindroth 2007) but do not appear to affect the growth or feeding of Lepidoptera (Hwang and Lindroth 1997, 1998), porcupine (Diner and others unpublished data) or elk (Bailey and others 2007; Wooley and others 2008). Although aspen CT have little to no detectable effects on the performance of the forest tent caterpillar (*Malacosoma disstria*), they were positively correlated with adult body mass of female parasitoids (*Compsilura concinnata*) utilizing tent caterpillars as hosts ($R^2 = 0.33$, $P < 0.001$, Holton and others 2003).

In contrast, other studies found that CT in bark tissues may act as a deterrent to consumers and endophytes. Bailey and others (2004) found that foraging by beaver (*Castor canadensis*) along a stream dominated by *Populus* (*P. fremontii*, *P. angustifolia* and their hybrids) resulted in selective felling of trees with low concentrations (<2%) of bark CT. Beaver selected 29% more *P. fremontii*, and 18% more F₁ hybrids than expected, suggesting that plant traits related to *Populus* hybridization (specifically concentrations of CT) might influence patterns of beaver herbivory. Experimentally, beavers were 12 times more likely to select a genotype with low bark concentrations of CT (0–2% dry weight) than a genotype with high concentrations of CT (>2% dry weight). These data show that beavers discriminated between closely related *Populus* species and their natural hybrids based, in part, on the

concentration of bark CT. Fungal endophyte infection is also negatively related to the concentration of CT in twig tissue (Bailey and others 2005). Concentrations of CT in twigs varied by less than 2% and yet that variation resulted in a 54% reduction in colonization by fungal endophytes. Thus, although there have been some deterrent associations of CT in *Populus* plant tissues (most notably within woody stem or twig tissues), overall, the effects of CT on associated herbivore fitness and performance or endophytes are inconclusive. These studies suggest that the effects of CT are likely species-, tissue-, and context-dependent. Associations between plants and the performance of herbivores or endophytes may also be complicated by correlations between CT and other phytochemical defenses such as phenolic glycosides (Basey and others 1988; Rehill and others 2005).

In contrast, the long-term effects of herbivore-induced CT are relatively strong and may link arthropods to belowground processes even after leaf abscission (Choudhury 1988; Findlay and others 1996; Chapman and others 2006). Induced resistance by increasing the production of CT may be a common means for dealing with herbivory in *Populus* species and clones (Havill and Raffa 1999; Arnold and Schultz 2002; Arnold and others 2004), contributing to large within-plant variation in CT. Recent work has quantified specific genetic mechanisms for the up-regulation of genes for plant secondary compounds after herbivory (Peters and Constabel 2002; Ralph and others 2006; Tsai and others 2006), demonstrating that induction is under genetic control. Induced expression of CT following herbivory can have long-term ecological consequences. Examples of post-senescent effects of herbivory in *Populus* spp. include studies by both Findlay and others (1996) and Schweitzer and others (2005), where herbivore-induced CT were maintained after the leaves senesced and abscised. Increased concentrations of CT (and nutrients) induced by herbivory were retained in leaf litter and significantly affected rates of litter decomposition; leaf litter that sustained herbivory decayed at much slower rates (20–34%) than leaves unaffected by foliar herbivory. Thus, induction by herbivory may be one understudied mechanism by which associated herbivores have lasting influences on ecosystems (Hunter 2001; Nykänen and Koricheva 2004; Chapman and others 2006).

Belowground Effects

Although the data are mixed for the effects of CT on aboveground processes, CT have repeatedly

been shown to impact ecosystem processes (C and N cycles specifically and the microbial communities that mediate them) either directly or indirectly. For example, disparate groups of organisms demonstrate significant relationships between community composition and concentrations of CT in *Populus* that link above- and belowground processes (Figure 3). Using phospholipid fatty acid (PLFA) biomarkers, Schweitzer and others (2007) found significant differences in microbial community composition in soils beneath *Populus* genotypes that varied in their expression of foliar CT. Although PLFA can discriminate among microbial community members only at coarse levels (fungi, gram-positive bacteria, gram-negative bacteria, and so on) they demonstrate that unique functional groups of diverse microorganisms occur beneath different tree genotypes that vary by relatively few

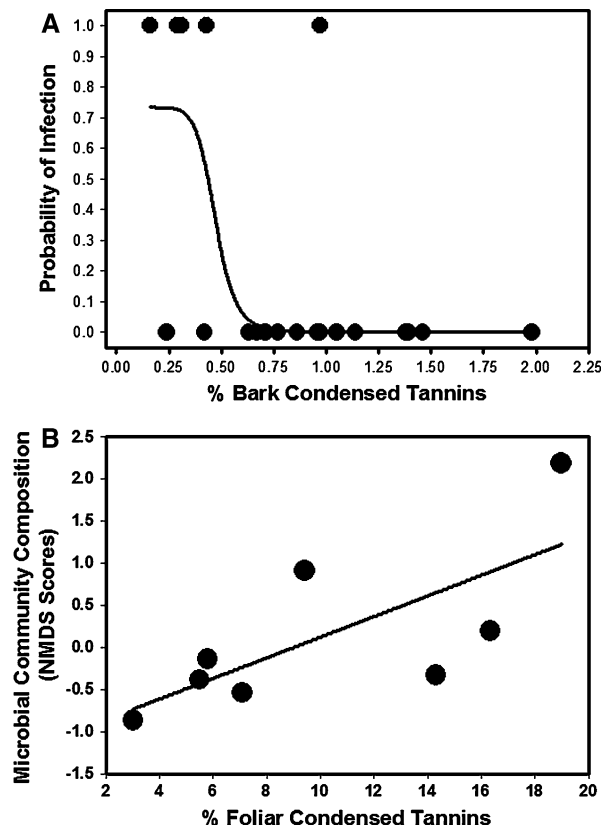


Figure 3. Across a *Populus* hybridizing complex, condensed tannins (CT) are related to the probability of endophyte infection (A), and soil microbial community composition (B; as measured by phospholipids fatty acid biomarkers—genotype means are presented). Non-metric multi-dimensional scaling (NMDS) ordination scores represent the Bray–Curtis distance measure of community composition (see Schweitzer and others 2007 for details). Figures and/or data modified from Bailey and others (2005) and Schweitzer and others (2007).

molecular and morphological traits (including concentration of CT). Furthermore, Madritch and others (2007a) found that herbivory by two dominant foliar herbivores (Gypsy moths and forest tent caterpillars) resulted in frass deposition that reflected the same concentration of CT in the foliage of the plant genotype the arthropod consumed. Deposition of that frass affected both belowground respiration and extracellular enzyme activity of microbial communities. Together these data suggest that at several levels, CT may have the ability to influence individual and community dynamics of microorganisms (*sensu* Field and Lettinga 1992; Bending and Read 1996; Kosola and others 2006).

Condensed tannins may link important above- and belowground processes by affecting the microbial communities that mediate nutrient cycles (primarily C and N), or by having a direct or indirect effect on C partitioning above- and belowground (Fischer and others 2006, 2007). For example, in both the field and in a common garden, Fischer and others (2006) found that tree types that have high foliar concentrations of CT also have higher fine root production than trees with low concentrations of CT in their foliage (Figure 4). Aboveground growth may also be reduced in genotypes of *Populus* that produce high concentrations of CT in foliar or root tissues (Fischer and others 2006; Donaldson and others 2006; Lowjewski 2007). Hence, either intrinsically, or through an external feedback that includes soil microorganisms (potentially including mycorrhizae; Gehring and others 2006) and soil N

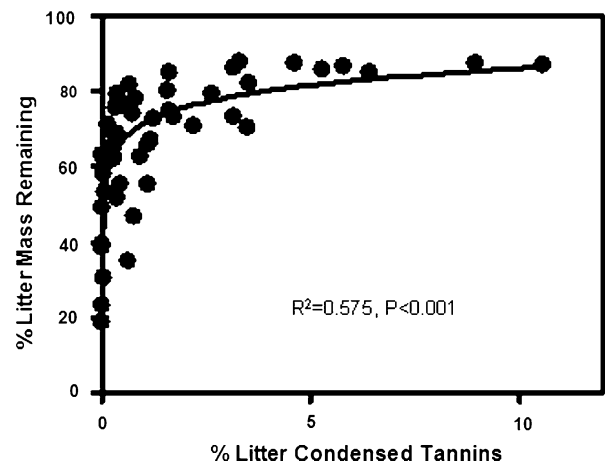


Figure 4. Litter mass remaining in *Populus tremuloides* is related to initial concentration of condensed tannins (CT) at time t correlated with percent mass remaining at time $t + 1$. Statistical results are from a log-linear regression, with untransformed data shown. $N = 50$ (5 genotypes, 2 nutrient treatments, 5 timesteps). Figures and data modified from Madritch and others (2006).

availability, CT may co-vary with plant C allocation above- and belowground in terrestrial ecosystems (Fischer and others 2007).

As described in recent reviews (Hättenschwiler and Vitousek 2000; Kraus and others 2004; Hättenschwiler and others 2005), data have long shown that CT in plant foliage and roots may strongly affect heterotrophic microbial communities, slowing rates of leaf litter decomposition and nutrient release. Terrestrially, CT in *Populus* have been shown to be negatively correlated with litter decomposition, explaining up to 76% of the variation in concentration of CT across a *Populus* hybridizing complex (Schweitzer and others 2004). Condensed tannins have also been found to be related to rates of decay within species. For example, Madritch and others (2006) found an overall negative relationship between the concentration of CT in initial leaf litter and decomposition rate in five *P. tremuloides* genotypes. Time series analysis (to examine the relationships of CT to mass loss during each stage of decomposition) indicated that 57% of the variation in mass remaining at the end of each timestep (that is, collection date) during a 1-year period was explained by variation in the concentration of CT at the beginning of that timestep (Figure 4). Although a general pattern of the impacts of CT on litter decay and nutrient release has been supported in multiple studies within the genus *Populus*, the direction and magnitude of the relationship varies with environmental context (that is, terrestrial or aquatic). Whereas few studies have experimentally manipulated quantitative or qualitative variation in CT to determine direct effects on ecosystem processes (but see Kraus and others 2003, 2004), if CT are consistently related to altered nutrient regimes, then feedback responses to either fitness or performance in plants (in either evolutionary time or over the lifetime of single long-lived organism) are likely.

In soils associated with *Populus*, as with other plant species, CT in plant tissues alter C and N mineralization and nitrification in the soil as well as whole-plant responses (*sensu* Hättenschwiler and Vitousek 2000; Kraus and others 2004; Nierop and others 2006, but see Castells and others 2004). Altered rates of net N mineralization and nitrification in soil were found in relation to successional dynamics in *Populus balsamifera* L. (balsam poplar) due to variation in litter CT between early and later successional species (*Alnus tenuifolia* Nutt. and *P. balsamifera*, respectively) in Alaska (Clein and Schimel 1995; Schimel and others 1996, 1998). However, the strength of the microbial response to variation in litter quality was dependent upon

molecular structure (Fierer and others 2001). In both the field and laboratory incubations, soils associated with stands of trees with greater inputs of CT (*Populus angustifolia* and backcross hybrids) have also been found to have lower annual rates of net N mineralization and nitrification than trees with smaller inputs of CT (*Populus fremontii*; Figure 5; Schweitzer and others 2004). Total input of CT in leaf litter (that is, leaf litter biomass multiplied by the concentration of CT) across stands accounted for 63% of the variation in net N mineralization in the field. In N-limited ecosystems, such changes in soil N availability may represent an important mechanism to conserve soil N that may feed back to affect plant fitness and performance (Northup and others 1998).

Thus, the role of CT as regulators of soil microbial communities, organic matter decomposition and subsequent mineralization and leaching processes to affect soil N availability and plant productivity could be highly adaptive (Northup and others 1998; Fischer and others 2006). Mechanisms by which CT in soils immobilize soil nutrients (either in microbial biomass or tannin complexes in the soil) may help prevent loss of soil nutrients through leaching (Schweitzer 2002). Further, nutrients bound by CT may be accessible only by mycorrhizal mutualisms with plants producing high concentrations of CT, allowing competitive exclusion of neighboring plants without similar mycorrhizae (see Northup and others 1998; Kraus and others 2003). Lastly, high fine root production in some *Populus* spp. allows rapid response and foraging for nutrients by plants producing high concentrations of CT, also resulting in competitive exclusion of plant neighbors with low root growth or low production of CT (Figure 5; Fischer and others 2006).

Aquatic Effects

Patterns of CT in aquatic systems are consistent with studies in terrestrial systems, although the adaptive mechanisms are more unclear. Studies across a broad array of plants have shown that CT can negatively influence macroinvertebrate communities, rates of litter decay and nutrient release in streams (Palm and Sanchez 1991; Campbell and Fuchshuber 1995, but see Ostrofsky 1997; Ardon and others 2006). Within *Populus*, macroinvertebrate community composition (Driebe and Whitham 2000; LeRoy and others 2006) can vary across species, with concentrations of CT explaining up to 55% of the variation in community composition (Whitham and others 2006). However, at the level of plant genotype, macroinvertebrate communities

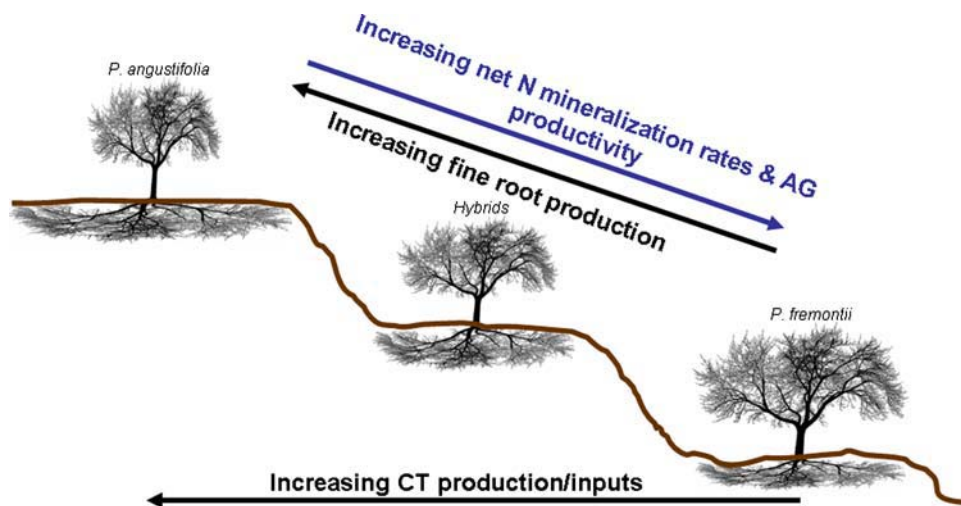


Figure 5. Schematic representations of the ecological effects of inputs of CT across geographic regions and a hybridizing complex, under field conditions. Expression of CT is highest in *P. angustifolia* genotypes, which are associated with low rates of net N mineralization, low aboveground (AG) productivity and high fine root production (compiled from Schweitzer and others 2004; Fischer and others 2006; Lowjewski 2007).

showed no response to variation in leaf litter CT (LeRoy and others 2007). Despite idiosyncratic effects on decomposer communities, studies across a hybridizing complex (Driebe and Whitham 2000; LeRoy and others 2006) and within species (LeRoy and others 2007; S.C. Wooley and R.L. Lindroth unpublished data) have consistently demonstrated that high concentrations of foliar CT and lignin in leaf litter slow leaf litter decomposition rates. Variation in initial concentration of CT can explain 38–97% of the variation in decay rates. In addition, nutrient availability during plant growth influences the rate of decomposition in aquatic systems, with CT explaining more than 40% of the variation in decomposition rate, but only among leaf litter with high nutrient concentrations (that is, litter from fertilized plants; S.C. Wooley and R.L. Lindroth unpublished data). These data suggest that although initial concentration of CT may be important to litter decay processes in streams, they may be less directly tied to specific populations of organisms or communities (LeRoy and others 2007), than has been observed in terrestrial studies (Bailey and others 2005, 2006; Bangert and others 2006; Schweitzer and others 2007).

Leaf litter decay rates vary similarly across terrestrial and aquatic habitats demonstrating common patterns in decay rate related to CT (Figure 6). Genotypes with large concentrations of initial CT decay more slowly than genotypes with low initial concentrations of CT, whether decomposing in the stream or in the adjacent riparian forest (Driebe

and Whitham 2000; Schweitzer and others 2004; LeRoy and others 2006, 2007; S.C. Wooley and R.L. Lindroth unpublished data). Overall, across genotypes in a hybridizing complex, CT (or CT:N) explains up to 97% of the variation in decay in both environments. Similarly, replicated genotypes of *P. tremuloides* decomposed terrestrially have decay rates that are negatively correlated with CT:N ($R^2 = 0.283$, $P < 0.001$; data from Madritch and others 2006). These studies demonstrate that across multiple scales (within a species, among crosstypes in a hybridizing complex, locally, regionally), foliar CT or the CT:N ratio, have predictable overall effects on rates of litter decay in streams or on land. Consistent effects of CT on leaf litter decomposition have been shown to influence rates of nutrient release, annual rates of net N mineralization and overall nutrient conservation in terrestrial ecosystems, which supports contentions that CT have important and predictable effects on ecosystem processes (*sensu* reviews in Hättenschwiler and Vitousek 2000; Kraus and others 2003).

FUTURE DIRECTIONS: FEEDBACKS TO PLANT FITNESS

Findings with *Populus* spp. demonstrate that there are strong genetic mechanisms regulating the expression of CT, which have predictable ecosystem consequences in both forests and streams. However, many issues must be addressed to place ecosystem science in the evolutionary framework

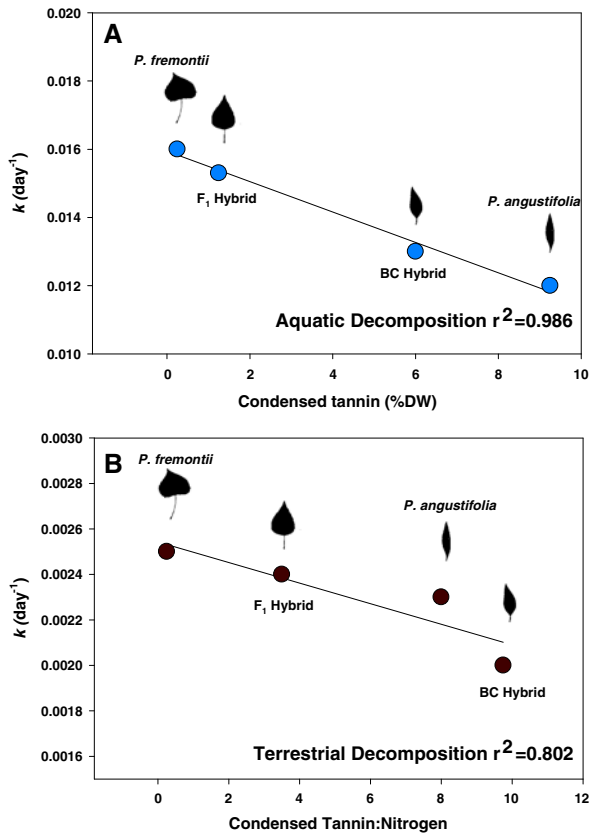


Figure 6. Exponential decomposition rate constants (k/d^{-1}) for *Populus* species and cross types (*P. fremontii*, *P. angustifolia* and their natural F₁ and backcross hybrids) within aquatic (**A**) and terrestrial (**B**) habitats. There is a negative relationship between k and the initial litter concentrations of condensed tannins (CT) in aquatic environments and initial mass ratios of condensed tannin to nitrogen ratio (CT:N) of litter in terrestrial environments. Leaf litter decomposes fastest when low in CT or with a low CT:N across aquatic and terrestrial environments. Figures modified from LeRoy and others (2006) (**A**) and Schweitzer and others (2004) (**B**).

that a “genes to ecosystems” perspective implies. The most important is the experimental demonstration that there are fitness consequences due to variation in CT. Evidence is growing that variation in CT can impact fitness and performance of the individual plant expressing that trait but to date this evidence is mostly anecdotal. For example, there is some evidence that CT can convey resistance to herbivores, which positively affects the survival of those plants with high concentrations of tannins, although the results of these studies are mixed (Whitham and others 2006). From a plant–soil perspective, the fitness consequences of CT are more evident. The literature indicates that soil microbial communities consistently respond to CT across environmental gradients: rates of nutrient

release and nutrient leaching are reduced, which ultimately results in the conservation of soil N. Feedbacks from these processes that affect plant fitness are likely. Clearly, much research effort will be required to experimentally address feedbacks to fitness implied by the data in *Populus*. Success in this endeavor, however, will have large implications for understanding evolutionary mechanisms in ecosystem ecology.

CONCLUSIONS

Several common themes emerge when taking the ‘genes-to-ecosystem’ approach based on the breadth of studies to date within *Populus* (described here). First, variation in production of CT has a clear genetic basis. The genetic basis of CT is apparent at the phylogenetic level suggesting a deep evolutionary history, but also at the individual level within species. Now that quantitative trait loci (QTL) have been identified for CT and are being merged with the *Populus* genome sequence to identify specific genes that code and regulate the production of CT, the potential to quantify the ecosystem phenotypes of CT are greater than ever before (Whitham and others 2008). Furthermore, the potential to develop knockout genotypes that reduce or eliminate CT while having the same genetic background as other individuals will greatly enhance our ability to clearly define the specific roles of CT in the individual, community and ecosystem as well as their effects on fitness. Second, the effects of CT appear to be strong belowground, suggesting a critical role for tannins in regulating nutrient dynamics outside of the traditional paradigm of consumer defense. As illustrated in Figure 1, the ecological effects of CT appear to be consistent on belowground and aquatic processes that may act to conserve available nutrients in nutrient limited ecosystems. Although much work is still required to understand the mechanistic details of CT in ecosystems, such as the role of structural variation of CT in ecological processes, the chemical fate of CT in soils, and the specific relationship between CT and microorganisms (including endosymbionts in consumers, mycorrhizal mutualists or free-living heterotrophs), the data in *Populus* suggest that CT have many adaptive roles. Specifically, the results using *Populus* as a model system suggest that the influence of CT on soil carbon and nitrogen dynamics may be widespread and have significant impacts on feedbacks between plant and soil processes within terrestrial ecosystems (Binkley and Giardina 1998). These studies suggest that an underappreciated adaptive

role of CT may lie in the nutrient regulation of soils as they integrate patterns across ecosystem boundaries (that is, between forests and streams) and between above- and belowground components of ecosystems.

ACKNOWLEDGEMENTS

We thank the extended Whitham, Lindroth, Hagerman, Hart and Keim lab groups for their support and feedback on these ongoing studies. Support was provided for various aspects of these projects from the U.S. National Science Foundation, including the Integrated Research Challenges in Environmental Biology and Frontiers in Integrated Biological Research programs (DEB-0078280, DEB-0425908) as well as NSF grants DEB-9707263, DEB-0074427, DEB-0344019, DEB-0743437, REU-DBI-0353915 and the U.S. Dept. of Agriculture, cooperative agreement 58-1932-6-634. We also thank the Ogden Nature Center and the Utah Department of Natural Resources for their support of our common garden studies, which have been crucial for separating the genetic and environmental effects of CT production.

REFERENCES

- Ardon M, Stallcup LA, Pringle CM. 2006. Does leaf quality mediate the stimulation of leaf breakdown by phosphorus in neotropical streams? *Freshwater Biol* 51:618–33.
- Arnold TM, Schultz JC. 2002. Induced sink strength as a prerequisite for induced tannin biosynthesis in developing leaves of *Populus*. *Oecologia* 130:585–93.
- Arnold T, Appel H, Patel V, Stocum E, Kavalier A, Schultz J. 2004. Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink–source model of plant defense. *New Phytol* 164:157–64.
- Athanasiadou S, Kyriazakis I. 2004. Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. *Proc Nutr Soc* 63:631–9.
- Ayres MP, Clausen TP, MacLean SF Jr, Redman AM, Reichardt PB. 1997. Diversity of structure and anti-herbivore activity in condensed tannins. *Ecology* 78:1696–712.
- Bailey JK, Schweitzer JA, Martinsen GD, Howe MA, Whitham TG. 2002. Beaver preference and plant species shifts: implications for exotic invasions. In: Proceedings from the 11th international conf. of exotic invasions of aquatic systems, pp 180–8.
- Bailey JK, Schweitzer JA, Rehill BJ, Lindroth RL, Keim P, Whitham TG. 2004. Beavers as molecular geneticists: a genetic basis to the foraging of an ecosystem engineer. *Ecology* 85:603–8.
- Bailey JK, Deckert R, Schweitzer JA, Rehill BJ, Lindroth RL, Gehring C, Whitham TG. 2005. Host-plant genetics affect hidden ecological players: links among *Populus*, condensed tannins and fungal endophyte infection. *Can J Bot* 83:356–62.
- Bailey JK, Wooley SC, Lindroth RL, Whitham TG. 2006. Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecol Lett* 9:78–85.
- Bailey JK, Irschick DJ, Schweitzer JA, Rehill BJ, Lindroth RL, Whitham TJ. 2007. Selective herbivory by elk results in rapid shifts in the chemical composition of aspen forests. *Biol Invas* 9:715–22.
- Bangert RK, Turek RJ, Rehill BJ, Wimp GM, Schweitzer JA, Allan GJ, Bailey JK, Martinsen GD, Keim P, Lindroth RL, Whitham TG. 2006. A genetic similarity rule determines arthropod community structure. *Mol Ecol* 15:1379–91.
- Barbehenn RV, Jones CP, Hagerman AE, Karonen M, Salminen J-P. 2006. Ellagitannins have greater oxidative activities than condensed tannins and galloyl glucoses at high pH: potential impact on caterpillars. *J Chem Ecol* 32:2253–67.
- Basaraba J, Starkey RL. 1966. Effect of plant tannins on decomposition of organic substances. *Soil Sci* 101:17–23.
- Basey JM, Jenkins SH, Busher PE. 1988. Optimal central-place foraging by beavers: tree-size selection in relation to defensive chemicals of quaking aspen. *Oecologia* 76:278–82.
- Bending GD, Read DJ. 1996. Effects of the soluble polyphenol tannic acid on the activities of ericoid and ectomycorrhizal fungi. *Soil Biol Biochem* 28:1595–602.
- Bhat TK, Singh B., Sharma OP. 1998. Microbial degradation of tannins—A current perspective. *Biodegradation* 9:343–57.
- Benoit RE, Starkey RL. 1968. Inhibition of decomposition of cellulose and some other carbohydrates by tannin. *Soil Sci* 105:291–6.
- Bernays EA, Cooper DG, Bilgener M. 1989. Herbivores and plant tannins. In: Begon M, Fitter AH, Ford ED, MacFadyen A, Eds. *Advances in ecological research*, vol 19. London: Academic Press. pp 263–302.
- Binkley D, Giardina C. 1998. Why do tree species affect soils? The Warp and Woof of tree-soil interactions. *Biogeochemistry* 42:89–106.
- Bradley RL, Titus BD, Preston CP. 2000. Changes to mineral N cycling and microbial communities in black spruce humus after additions of (NH₄)₂SO₄ and condensed tannins extracted from *Kalmia angustifolia* and balsam fir. *Soil Biol Biochem* 32:1227–40.
- Bryant JP, III Chapin S, Klein DR. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40:357–68.
- Butler LG. 1989. Effects of condensed tannin on animal nutrition. In: Hemingway RW, Karchesy JJ, Eds. *Chemistry and significance of condensed tannins*. NY: Plenum Press. pp 391–416.
- Campbell IC, Fuchshuber L. 1995. Polyphenols, condensed tannins, and processing rates of tropical and temperate leaves in an Australian stream. *J N Am Benthol Soc* 14:174–82.
- Castells E, Peñuelas J, Valentine DW. 2004. Are phenolic compounds released from the Mediterranean shrub *Cistus albidus* responsible for change in N cycling in siliceous and calcareous soils? *New Phytol* 162:187–95.
- Cates RG, Rhoades DF. 1977. Patterns in the production of anti-herbivore chemical defenses in plant communities. *Biochem Syst Ecol* 5:185–93.
- Chapman SC, Schweitzer JA, Whitham TG. 2006. Herbivory differentially alters plant litter dynamics of evergreen and deciduous trees. *Oikos* 114:566–74.
- Choudhury D. 1988. Herbivore induced changes in leaf-litter resource quality: a neglected aspect of herbivory in ecosystem nutrient dynamics. *Oikos* 51:389–93.
- Clausen TP, Provenza FD, Burritt EA, Reichardt PB, Bryant JP. 1990. Ecological implications of condensed tannin structure: a case study. *J Chem Ecol* 16:2381–92.

- Clein JS, Schimel JP. 1995. Nitrogen turnover and availability during succession from alder to poplar in Alaskan taiga forests. *Soil Biol Biochem* 27:743–52.
- Close DC, McArthur C. 2002. Rethinking the role of many plant phenolics—protection from photodamage not herbivores? *Oikos* 99:166–72.
- Conner JK, Hartl DL. 2004. *A Primer of ecological genetics*. Sunderland: Sinauer.
- Dalzell SA, Shelton SM. 2002. Genotypic variation in proanthocyanidin status of *Leucaena* genus. *J Agric Sci* 138:209–20.
- Dixon RA, Xie DY, Sharma SB. 2005. Proanthocyanidins—a final frontier in flavinoid research? *New Phytol* 165:9–28.
- Donaldson JR, Lindroth RL. 2004. Cottonwood leaf beetle (Coleoptera: Chrysomelidae) performance in relation to variable phytochemistry in juvenile aspen (*Populus tremuloides* Michx.). *Environ Entomol* 33:1505–11.
- Donaldson JR, Lindroth RL. 2007. Genetics, environment, and G × E interactions determine efficacy of chemical defense in trembling aspen. *Ecology* 88:729–39.
- Donaldson JR, Stevens MT, Barnhill HR, Lindroth RL. 2006. Age-related shifts in leaf chemistry of clonal aspen. *J Chem Ecol* 32:1415–29.
- Driebe EM, Whitham TG. 2000. Cottonwood hybridization affects tannin and nitrogen of leaf litter and alters decomposition. *Oecologia* 123:99–107.
- Dungey HS, Potts BM, Whitham TG, Li H.-L. 2000. Plant genetics affects arthropod community richness and composition: evidence from a synthetic eucalypt hybrid population. *Evolution* 54:1938–46.
- Fahey Jr GC, Jung HG. 1989. Phenolic compounds in forages and fibrous feedstuffs. In: Cheeke PR, Ed. *Toxicants of plant origin*, vol IV, Phenolics. Florida: CRC Press, Inc. pp 123–90.
- Feeny PP. 1968. Effect of oak leaf tannins on larval growth of the winter moth *Operophtera brumata*. *J Insect Physiol* 14:805–17.
- Feeny PP. 1969. Inhibitory effects of oak leaf tannins on the hydrolysis of trypsin. *Phytochemistry* 8:2119–26.
- Feeny PP. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* 51:565–81.
- Field JA, Lettinga G. 1992. Toxicity of tannic compounds to microorganisms. In: Hemingway RW, Laks PE, Eds. *Plant polyphenols. Synthesis, properties, significance*. New York: Plenum Press. pp 673–92.
- Fierer N, Schimel JP, Cates RG, Zou Z. 2001. The influence of balsam poplar tannin fractions on carbon and nitrogen dynamics in Alaskan taiga floodplain soils. *Soil Biol Biochem* 33:1827–39.
- Findlay S, Carreiro M, Krischik V, Jones CG. 1996. Effects of damage to living plants on leaf litter quality. *Ecol Appl* 6:269–75.
- Fischer DG, Hart SC, Rehill BJ, Lindroth RL, Keim P, Whitham TG. 2006. Do high tannin leaves require more roots? *Oecologia* 149:668–75.
- Fischer DG, Hart SC, LeRoy CJ, Whitham TG. 2007. Variation in below-ground carbon fluxes along a *Populus* hybridization gradient. *New Phytol* 176:415–25.
- Forkner RE, Marquis RJ, Lill JT. 2004. Feeny revisited: condensed tannins as anti-herbivore defences in leaf-chewing herbivore communities of *Quercus*. *Ecol Entomol* 29:174–87.
- Gallardo A, Merino J. 1992. Nitrogen immobilization in leaf litter at two mediterranean ecosystems of SW Spain. *Biogeochemistry* 15:213–28.
- Gehring CA, Mueller RC, Whitham TG. 2006. Environmental and genetic effects on the formation of ectomycorrhizal and arbuscular mycorrhizal associations in cottonwoods. *Oecologia* 149:158–64.
- Hagerman AE. 1992. Tannin–protein interactions. *ACS Symp Ser* 506:236–47.
- Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC, McArthur C. 1992. Tannin chemistry in relation to digestion. *J Range Manage* 45:57–62.
- Harding SA, Jiang H, Jeong ML, Casado FL, Lin H-W, Tsai C-J. 2005. Functional genomics analysis of foliar condensed tannin and phenolic glycoside regulation in natural cottonwood hybrids. *Tree Physiol* 25:1475–86.
- Haslam E. 1988. Plant polyphenols (syn. vegetable tannins) and chemical defense—a reappraisal. *J Chem Ecol* 14:1789–806.
- Hättenschwiler S, Vitousek PM. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol Evol* 15:238–43.
- Hättenschwiler S, Tiunov AI, Scheu S. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. *Annu Rev Evol Syst* 36:191–218.
- Havill NP, Raffa KF. 1999. Effects of eliciting treatment and genotypic variation on induced resistance in *Populus*: impacts on gypsy moth development and feeding behavior. *Oecologia* 120:295–303.
- Hemming JDC, Lindroth RL. 1995. Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moths and forest tent caterpillars. *Oecologia* 103:79–88.
- Holton MK, Lindroth RL, Nordheim EV. 2003. Foliar quality influences tree–herbivore–parasitoid interactions: effects of CO₂, O₃ and plant genotype. *Oecologia* 137:233–44.
- Horner JD, Gosz JR, Cates RG. 1988. The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. *Am Nat* 132:869–83.
- Hunter MD. 2001. Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. *Agric Forest Entomol* 3:77–84.
- Hwang S-Y, Lindroth RL. 1997. Clonal variation in foliar chemistry of aspen: effects on gypsy moths and forest tent caterpillars. *Oecologia* 111:99–108.
- Hwang S-Y, Lindroth RL. 1998. Consequences of clonal variation in aspen phytochemistry for late season folivores. *Écoscience* 5:508–16.
- Iason GR, Vilalba JJ. 2006. Behavioral strategies of mammal herbivores against plant secondary compounds: the avoidance–tolerance continuum. *J Chem Ecol* 32:1115–32.
- Kanerva S, Kitunen V, Kiikkilä O, Loponen J, Smolander A. 2006. Response of soil C and N transformations to tannin fractions originating from Scots pine and Norway spruce needles. *Soil Biol Biochem* 38:1364–74.
- Kasurinen A, Keinänen MM, Kaipainen S, Nilsson L-O, Vapaavuori E, Kontro MH, Holopainen T. 2005. Below-ground response of silver birch trees exposed to elevated CO₂ and O₃ for three growing seasons. *Global Change Biol* 11:1167–79.
- Kearsley MJC, Whitham TG. 1998. The developmental stream of cottonwoods affects ramet growth and resistance to herbivory by galling aphids. *Ecology* 79:178–91.
- Kosola KR, Parry D, Workmaster BAA. 2006. Responses of condensed tannin in Poplar roots to fertilization and gypsy moth defoliation. *Tree Physiol* 26:1607–11.

- Kraus TEC, Dahlgren RA, Zasoski RJ. 2003. Tannins in nutrient dynamics of forest ecosystems—a review. *Plant Soil* 256:41–66.
- Kraus TEC, Zasoski RJ, Dahlgren RA, Horwath WR, Preston CM. 2004. Carbon and nitrogen dynamics in a forest soil amended with purified tannins from different plant species. *Soil Biol Biochem* 36:309–21.
- Lawrence R, Potts BM, Whitham TG. 2003. Relative importance of plant ontogeny, host genetic variation, and leaf age for a common herbivore. *Ecology* 84:1171–8.
- LeRoy CJ, Whitham TG, Keim P, Marks JC. 2006. Plant genes link forests and streams. *Ecology* 87:255–61.
- LeRoy CJ, Wooley SC, Whitham TG, Marks JC. 2007. Within-species variation in foliar chemistry influences leaf-litter decomposition in a Utah river. *J N Am Benthol Soc* 26:426–38.
- Lindroth RL, Hwang S-Y. 1996. Clonal variation in foliar chemistry of quaking aspen (*Populus tremuloides* Michx.). *Biochem Syst Ecol* 24:357–64.
- Lindroth RL, Kinney KK, Platz CL. 1993. Responses of deciduous trees to elevated atmospheric CO₂: productivity, phytochemistry and insect performance. *Ecology* 74:763–77.
- Lindroth RL, Roth S, Kruger EL, Volin JC, Koss PA. 1997. CO₂-mediated changes in aspen chemistry: effects on gypsy moth performance and susceptibility to virus. *Global Change Biol* 3:279–89.
- Lindroth RL, Donaldson JR, Stevens MT, Gusse AC. 2007. Browse quality in quaking aspen (*Populus tremuloides*): effects of genotype, nutrients, defoliation, and coppicing. *J Chem Ecol* 33:1049–64.
- Liu L, King JS, Giardina CP. 2005. Effects of elevated concentrations of atmospheric CO₂ and tropospheric O₃ on leaf litter production and chemistry in trembling aspen and paper birch communities. *Tree Physiol* 25:1511–22.
- Lowjewski NR. 2007. Genetic determination of aboveground net primary productivity in a riparian foundation tree species. Master's Thesis, Northern Arizona University.
- Madritch MD, Hunter MD. 2002. Phenotypic diversity influences ecosystem functioning in an oak sandhills community. *Ecology* 83:2084–90.
- Madritch MD, Donaldson JR, Lindroth RL. 2006. Genetic identity of *Populus tremuloides* litter influences decomposition and nutrient release in a mixed forest stand. *Ecosystems* 9:528–37.
- Madritch MD, Donaldson JR, Lindroth RL. 2007. Canopy herbivory can mediate the influence of plant genotype on soil processes through frass deposition. *Soil Biol Biochem* 39:1192–201.
- Madritch MD, Jordan LM, Lindroth RL. 2007. Interactive effects of condensed tannin and cellulose additions on soil respiration. *Can J Forest Res* 37:2063–7.
- Mansfield JL, Curtis PS, Zak DR, Pregitzer KS. 1999. Genotypic variation for condensed tannin production in trembling aspen (*Populus tremuloides*, Salicaceae) under elevated CO₂ and in high- and low-fertility soil. *Am J Bot* 86:1154–9.
- Marles MAS, Ray H, Gruber MY. 2003. New perspectives on proanthocyanidin biochemistry and molecular regulation. *Phytochemistry* 64:357–83.
- Nierop KGJ, Preston CM, Verstraten JM. 2006. Linking the B ring hydroxylation pattern of condensed tannins to C, N and P mineralization: a case study using four tannins. *Soil Biol Biochem* 38:2794–802.
- Northup RR, Dahlgren RA, McColl JG. 1998. Polyphenols as regulators of plant–litter–soil interactions in Northern California's pygmy forest: a positive feedback? *Biogeochemistry* 42:189–220.
- Nykänen H, Koricheva J. 2004. Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos* 104:247–68.
- O'Reilly-Wapstra JM, Potts BM, McArthur C, Davies NW, Tilyard P. 2005. Inheritance of resistance to mammalian herbivores and of plant defensive chemistry in a *Eucalyptus* species. *J Chem Ecol* 31:357–75.
- Osier TL, Lindroth RL. 2001. Effects of genotype, nutrient availability and defoliation on aspen phytochemistry and insect performance. *J Chem Ecol* 27:1289–313.
- Osier TL, Lindroth RL. 2004. Long-term effects of defoliation on quaking aspen in relation to genotype and nutrient availability: plant growth, phytochemistry and insect performance. *Oecologia* 139:55–65.
- Osier TL, Lindroth RL. 2006. Genotype and environment determine allocation to and costs of resistance in quaking aspen. *Oecologia* 148:293–303.
- Ostrofsky ML. 1997. Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. *J N Am Benthol Soc* 16:750–9.
- Palm CA, Sanchez PA. 1991. Nitrogen release from the leaves of some tropical legumes as affected by their lignin and polyphenolic contents. *Soil Biol Biochem* 23:83–88.
- Peters DJ, Constabel JP. 2002. Molecular analysis of herbivore-induced condensed tannin synthesis: cloning and expression of dihydroflavonol reductase from trembling aspen (*Populus tremuloides*). *Plant Journal* 32:701–12.
- Ralph S, Oddy C, Cooper D, others. 2006. Genomics of hybrid poplar (*Populus trichocarpa* × *P. deltoides*) interacting with forest tent caterpillars (*Malacosoma disstria*): normalized and full-length cDNA libraries, expressed sequence tags, and a cDNA microarray for the study of insect-induced defences in poplar. *Mol Ecol* 15:1275–97.
- Rehill BJ, Clauss A, Wiczorek L, Whitham TG, Lindroth RL. 2005. Foliar phenolic glycosides from *Populus fremontii*, *Populus angustifolia*, and their hybrids. *Biochem Syst Ecol* 33:125–31.
- Rehill BJ, Whitham TG, Martinsen GD, Schweitzer JA, Bailey JK, Lindroth RL. 2006. Developmental trajectories in cottonwood phytochemistry. *J Chem Ecol* 32:2269–85.
- Rhodes DF, Cates RG. 1976. Toward a general theory of plant antiherbivores chemistry. *Recent Adv Phytochem* 10:168–213.
- Robbins CT, Hanley TA, Hagerman AE, Hjeljord O, Baker DL, Schwartz CC, Mautz WW. 1987. Role of tannins in defending plants against ruminants: reduction in protein availability. *Ecology* 68:98–107.
- Scalbert A. 1991. Antimicrobial properties of tannins. *Phytochemistry* 30:3875–83.
- Schimel JP, Van Cleve K, Cates RG, Clausen TP, Reichardt PB. 1996. Effects of balsam poplar (*Populus balsamifera*) tannins and low molecular-weight phenolics on microbial activity in taiga floodplain soil: implications for changes in N cycling during succession. *Can J Bot* 74:84–90.
- Schimel JP, Cates RG, Ruess R. 1998. The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga. *Biogeochemistry* 42:221–34.
- Schultz JC. 1989. Tannin–insect interactions. In: Hemingway RW, Karchesy JJ, Eds. *Chemistry and significance of condensed tannins*. NY: Plenum Press. pp 416–33.

- Schweitzer JA. 2002. Genetic variation associated with natural hybridization in Cottonwood affects riparian structure and function. Dissertation, Northern Arizona University.
- Schweitzer JA, Bailey JK, Rehill BJ, Hart SC, Lindroth RL, Keim P, Whitham TG. 2004. Genetically based trait in dominant tree affects ecosystem processes. *Ecol Lett* 7:127–34.
- Schweitzer JA, Bailey JK, Hart SC, Wimp GM, Chapman SC, Whitham TG. 2005. The interaction of plant genotype and herbivory decelerate leaf litter decomposition and alter nutrient dynamics. *Oikos* 110:133–45.
- Schweitzer JA, Bailey JK, Bangert RK, Hart SC, Whitham TG. 2007. The role of plant genetic variation in determining above- and belowground microbial communities. In: Bailey MJ, Lilley AK, Timms-Wilson TM, Spencer-Phillips, Eds. *Microbial ecology of aerial plant surfaces*. Wallingford, UK: CABI Publishing.
- Stevens ML, Lindroth RL. 2005. Induced resistance in the indeterminate growth of aspen (*Populus tremuloides*). *Oecologia* 145:298–306.
- Stout RJ. 1989. Effects of condensed tannins on leaf processing in mid-latitude and tropical streams: a theoretical approach. *Can J Fish Aqua* 46:1097–106.
- Swain T. 1979. Tannins and lignins. In: Rosenthal GA, Janzen DH, Eds. *Herbivores: their interactions with secondary plant metabolites*. New York: Academic Press. pp 657–82.
- Treseder KL, Vitousek PM. 2001. Potential ecosystem-level effects of genetic variation among populations of *Metrosideros polymorpha* from a soil fertility gradient in Hawaii. *Oecologia* 126:266–75.
- Tsai C-J, Harding SA, Tschaplinski TJ, Lindroth RL, Yuan Y. 2006. Genome-wide analysis of the structural genes regulating defense phenylpropanoid metabolism in *Populus*. *New Phytol* 172:47–52.
- Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, others. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. and Gray). *Science* 313:1596–604.
- Verkaik E, Jongkind A, Berendse F. 2006. Short-term and long-term effects of tannins on nitrogen mineralisation and litter decomposition in kauri (*Agathis australis* (D. Don) Lindl.) forests. *Plant Soil* 287:337–45.
- Waltz AM, Whitham TG. 1997. Plant development affects arthropod communities: opposing impacts of species removal. *Ecology* 78:2133–44.
- Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf EV, Allan GD, DiFazio SP, Potts BM, Fischer DG, Gehring CA, Lindroth RL, Marks J, Hart SC, Wimp GM, Wooley SC. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. *Nat Rev Genet* 7:510–23.
- Whitham TG, DiFazio SP, Schweitzer JA, Shuster SM, Allan JG, Bailey JK, Woolbright SA. 2008. Extending genomics to natural communities and ecosystems. *Science* 320:492–5.
- Woolbright S. 2001. Genetic analyses of a synthetic population of hybrid cottonwoods with implications for community-level processes. Master's thesis, Northern Arizona University.
- Wooley SC, Walker SC, Vernon J, Lindroth RL. 2008. Aspen decline, aspen chemistry, and elk herbivory: are they linked? *Rangelands* 30:17–21.
- Xie D-Y, Sharma SB, Paiva NL, Ferreira D, Dixon RA. 2003. Role of anthocyanidin reductase, encoded by BANYULS in plant flavonoid biosynthesis. *Science* 299:396–9.