

Genetic-based plant resistance and susceptibility traits to herbivory influence needle and root litter nutrient dynamics

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Summary

1. It is generally assumed that the same factors drive the decomposition of both litter and roots and that nutrient release from litter and roots is synchronized. However, few studies have explicitly tested these assumptions, and no studies have examined whether plant genetics (i.e. plant susceptibility to herbivory) could affect these relationships.

2. Here we examine the effects of herbivore susceptibility and resistance on needle and fine root litter decomposition of piñon pine, *Pinus edulis*. The study population consists of individual trees that are either susceptible or resistant to herbivory by the piñon needle scale, *Matsucoccus acalyptus*, or the stem-boring moth, *Dioryctria albobittella*. Genetic analyses and long-term experimental removals and additions of these insects to individual trees have identified trees that are naturally resistant or susceptible to *M. acalyptus* and *D. albobittella*. In addition, these herbivores increase litter chemical quality and alter soil microclimate, both of which mediate decomposition in ecosystems.

3. The effects of herbivore susceptibility and resistance on needle litter mass and phosphorus (P) loss, when significant, are largely mediated by herbivore-induced changes to microclimate. But the effects of herbivore susceptibility and resistance on root litter nitrogen (N) and P retention, and needle litter N retention, are largely governed by herbivore-induced changes to litter chemical quality. Whether a particular tree was resistant or susceptible to herbivores exerted a large influence on net nutrient release, but the direction of herbivore influence varied temporally.

4. The controls on decomposition vary between herbivore-susceptible and herbivore-resistant phenotypes. This suggests that understanding decomposition and nutrient retention in some ecosystems may require considering the effects of herbivores on above- and below-ground processes and how these effects may be governed by plant genetics.

5. *Synthesis.* Because so few studies have attempted to quantify genetic components of ecosystem processes, the integration of ecosystem ecology with population genetics has the potential to place ecosystem science within a genetic and evolutionary framework. Using field trials of known genetic composition, ecosystem scientists may use quantitative genetics techniques to explore ecosystem traits just as population geneticists have used these techniques to explore traditional traits such as resistance to insects.

Key-words: decomposition, genotype, herbivory, insects, needle, nitrogen, nutrient cycling, phosphorus, root, semi-arid

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Introduction

Plant genetics can directly influence ecosystem processes by causing changes in the quality of plant inputs to soils (e.g. differential sensitivity of different genotypes

to UV degradation, and temperature regulated processes). They can also have an indirect influence on ecosystem processes by affecting other species, which in turn affect ecosystem processes (e.g. resistance to herbivory, which affects productivity and litter quality). Thus, direct genetic effects alter ecosystem processes independent of other species or biotic interactions, whereas indirect genetic effects act through other species to affect ecosystem processes (see interspecific indirect genetic effects, IIGEs; Shuster *et al.* 2006). Both direct and indirect genetic effects are likely to be important in defining ecosystem processes (e.g. Treseder & Vitousek 2001; Madritch *et al.* 2006; Whitham *et al.* 2006), but few studies have contrasted their relative effects on ecosystem processes. In the present paper, we do not discriminate between these two pathways, but rather emphasize that regardless of the direct or indirect genetic contribution, the overall effects on ecosystems are ultimately genetic in origin.

Insect herbivores are pervasive in forested ecosystems, and plant genetic variation in herbivore resistance may differentially impact above- and below-ground processes such as nutrient cycling (Wardle *et al.* 2004). In addition, herbivory may regulate the feedback between plant input quality and nutrient cycling (Stadler *et al.* 2001; Wardle *et al.* 2001, 2002, 2004). However, interpretation of this regulation may be complicated by the opposing effects insect susceptibility can have on above- and below-ground nutrient inputs and release (Holland *et al.* 1996). For instance, leaf and root litter decomposition can have contrasting effects on ecosystem nutrient retention (Ostertag & Hobbie 1999; Gholz *et al.* 2000), a pattern that may be magnified by the effects of herbivore susceptibility on root and needle quality or on microclimate. Furthermore, above-ground leaf litter decomposition is controlled primarily by litter chemistry at the local level and by climate at the regional level (Meentemeyer 1978; Aerts 1997). The controls on root decomposition may differ from the controls on leaf litter decomposition (Silver & Miya 2001). Therefore, in this paper we investigate both above- and below-ground nutrient processes to help resolve the contrasting accelerating vs. decelerating effects of herbivory on decomposition (Wardle *et al.* 2001, 2002). Herbivore resistance and susceptibility that has a genetic origin allows us to investigate more explicitly the different impacts of herbivores on above- and below-ground nutrient fluxes.

The effects of herbivore susceptibility on nutrient cycling may be mediated not only by the quality of inputs, but also by the location of those inputs (e.g. above- vs. below-ground) and by the overall climate of the ecosystem (e.g. arid vs. mesic). In semi-arid environments, where moisture, more so than litter quality, may determine decomposition dynamics, we expected herbivore susceptibility effects on soil microclimate to be an important driver of decomposition. Specifically, herbivore susceptibility effects on litter quality should drive decomposition below ground where moisture is

less limiting than it is above ground. Additionally, herbivore susceptibility could influence decomposition by affecting litter layer moisture, and this influence should be more pronounced where moisture is most limiting, namely above ground. Thus, we predicted that: (i) herbivore susceptibility would have a larger impact on decomposition of exposed, above-ground needle litter than on buried root litter, and (ii) the effect of susceptibility on litter chemical quality would increase decomposition of the buried, below-ground root litter to a greater extent than the exposed above-ground needle litter. To test these general predictions, we measured the effects of herbivore susceptibility and resistance on litter decomposition, nutrient inputs and release over a 2-year period in a semi-arid ecosystem.

Methods

STUDY SITE AND CLIMATE

We conducted this study in a piñon-juniper woodland (35°22' N, 111°33' W) located adjacent to Sunset Crater National Monument, 30 km north-east of Flagstaff, AZ, USA. The site is *c.* 2100 m above sea level, and the soils in this region are well-drained and nutrient-poor members of USDA Soil Taxonomic subgroup Typic Ustorthents. Thirty-year means of precipitation and air temperature are 432 mm and 8.6 °C, respectively. Piñon pines represent 80% of the woody cover at the site. This study was conducted over a 2-year period that included a subaverage precipitation year (2001) and the driest year (2002) in recorded history in the region (Mueller *et al.* 2005).

Herbivory of piñon pines, *Pinus edulis*, by the piñon needle-scale, *Matsucoccus acalyptus*, and the stem-boring moth, *Dioryctria albobittella*, has been monitored for over 22 years at this site. Moth-susceptible (shrub-like, heavily attacked, mature trees with a closed architecture) and resistant (upright, lightly attacked mature trees with an open architecture) phenotypes were selected in 1982 (Whitham & Mopper 1985). Similarly, scale-susceptible (heavily infested chlorotic juvenile trees that have abscised most of their needles so that only the current 1–2 years of needle cohorts remain) and resistant (few or no scales with all 7 years of needle cohorts present) phenotypes were selected in 1985 (Cobb & Whitham 1993). Within each resistant and susceptible category, trees were selected at random (i.e. a stratified random sample), in which both tree categories grow interspersed at the same study site (*c.* 125 ha). Individual trees served as the units of replication in this study. Although a detailed analysis of the spatial distribution of susceptible and resistant trees has not been conducted, it is common to find resistant and susceptible trees growing immediately adjacent to one another (*moths*, Whitham & Mopper 1985; Gehring & Whitham 1991; Cobb *et al.* 1997, 2002; *scales*, Del Vecchio *et al.* 1993; Gehring *et al.* 1997). In other words, the probability that an individual tree's nearest

neighbour is resistant or susceptible is independent of whether it is susceptible or resistant itself. Thus, it is unlikely that microclimate or microsite determines whether a tree is resistant or susceptible to herbivores. For each herbivore, resistant and susceptible phenotypes were matched for relative size (i.e. age) so there would be no systematic differences in ontogeny that might influence plant–herbivore interactions (Kearsley & Whitham 1989). As moths attack only reproductively mature trees bearing female cones (at this site > 46 years; Ruel & Whitham 2002), and scale insects generally attack only juvenile trees ≤ 2 m in height, moths and scales do not co-occur on our study trees. Because most of the scale trees have not matured, we do not yet know if scale-susceptible trees grow into moth-susceptible trees.

Differential insect survival rates, long-term presence or absence of these insects on adjacent trees, and/or allozyme genetic analysis has enabled trees to be categorized as genetically resistant (hereafter ‘resistant’ trees) or susceptible (hereafter ‘susceptible’ trees) to these herbivores (Mopper *et al.* 1991b; Cobb & Whitham 1993; Gehring *et al.* 1997). Susceptible moth and scale trees that have had these insects experimentally removed have recovered to resemble resistant phenotypes demonstrating that these insects are responsible for their respective changes in tree morphology. Previous experiments also argue that microsite differences are not responsible for the observed phenotypic differences (e.g. resistance to insects, architecture, and interactions with mycorrhizae) because the experimental removal of these herbivores from susceptible trees results in the release of these traits to resemble resistant trees (moths, Whitham & Mopper 1985; Gehring & Whitham 1991; Cobb *et al.* 1997, 2002; scales, Del Vecchio *et al.* 1993; Gehring *et al.* 1997). Furthermore, resistant and susceptible trees grow intermixed in close proximity and genetic analyses of moth resistant and susceptible trees show that they are genetically differentiated (Mopper *et al.* 1991a). To avoid other potentially confounding variables such as competition and cross transfer of litter, we selected trees growing in the open. At this site piñon roots extend a maximum of two canopy widths past their drip line (C. A. Gehring, unpublished data). The open spaces between trees are devoid of grasses and support few forbs. Woody vegetation at the site is dominated by piñon pine (*Pinus edulis*), one-seeded juniper (*Juniperus monosperma*), and the shrub apache plume (*Fallugia paradoxa*).

Herbivory by both insects is chronic (i.e. continuous and at a high level). Needle-scale infestation significantly reduces canopy leaf area index, while it increases soil moisture, soil temperature, and litter chemical quality (Table 1; Chapman *et al.* 2003; Classen *et al.* 2005). Stem-boring moth infestation increases needle litter chemical quality and precipitation penetration to the soil surface (Table 1; Chapman *et al.* 2003; Classen *et al.* 2005). Moth and scale infestation reduces mycorrhizal colonization and root biomass and alters

microbial communities (Del Vecchio *et al.* 1993; Gehring *et al.* 1997; Kuske *et al.* 2003; Classen *et al.* 2006; Classen *et al.* 2007). Because much of this work was conducted on individual trees that have small canopies, not in a closed canopy forest, we were unable to use the same trees for all the data collected without causing significant damage to the tree; however, all the trees used in the experiment were part of the initial study population described above and were randomly selected from that population. We used three different groups of moth trees and five different groups of scale trees in order to conduct all the work that follows (Table 2). Whenever possible, measurements were taken on the same group of trees.

MICROCLIMATE AND CROWN ARCHITECTURE

Because microclimate often regulates decomposition and nutrient cycling in arid environments, we measured volumetric soil moisture (15–30 cm), maximum soil temperature (5 cm and 15 cm), leaf area index (LAI), and precipitation throughfall beneath moth-susceptible, moth-resistant, scale-susceptible and scale-resistant trees. These data were collected under the core group of study trees described here (in combination with needle litter decomposition bags) and measurements were taken over the course of this experiment (see Classen *et al.* 2005 for details). Data from this companion study are summarized in Table 1.

CHEMICAL QUALITY

To quantify how insect infestation altered nutrient inputs, we measured needle litter and root litter carbon (C), nitrogen (N), phosphorus (P), lignin, and needle condensed tannins. Samples were ground on a Wiley Mill through a 40-mesh (< 425 µm) screen (A. Thomas Co., Philadelphia, PA, USA). Subsamples were analysed for total C and N using a Carlo-Erba Model 2500 CHN analyser (Milan, Italy). Total Kjeldahl N and total Kjeldahl P were determined using a modified micro-Kjeldahl digestion (Parkinson & Allen 1975) and analysed for N and P concentrations on a Lachat Flow-Injection Analyser (Lachat Instruments, Inc., Loveland, CO, USA). Lignin concentrations were determined using a modification of the acetyl-bromide procedure (Iiyama & Wallis 1990; Chapman *et al.* 2003). Needle condensed tannin concentrations were determined using the butanol-HCl method (Porter *et al.* 1986). Samples were ashed at 550 °C and data are shown on an ash-free oven-dry mass basis.

Many scale insect carcasses fell off infested needles during drying and handling, and no effort was made to remove remaining scale tissue from needles prior to chemical analysis. A survey of 30 needles from each of 20 infested trees revealed an average of 22 scale insects per needle with a total mass of 0.47 mg. Based on analysis of scale N concentration (c. 50 mg g⁻¹ dry mass), we estimate that inclusion of the scales increased

Table 1. Herbivore susceptibility and resistance effects on soil volumetric water content (VWC), soil temperature, leaf area index (LAI), canopy interception, and root and needle litter input and chemistry. Within rows and tree type (moth and scale), contrasting letters denote significant differences ($P \leq 0.05$)

	Moth		Scale	
	Resistant	Susceptible	Resistant	Susceptible
				
Soil microclimate				
Volumetric water content 15-30 cm*	5.4 ^a	4.1 ^a	4.7 ^x	6.4 ^y
Temperature max 5 cm*	22.4 ^a	21.4 ^a	22.2 ^x	27.9 ^y
Temperature max 15 cm*	19.1 ^a	19.4 ^a	19.7 ^x	22.2 ^y
Canopy				
Leaf area index*	4.2 ^a	3.8 ^a	2.3 ^x	1.4 ^y
Canopy interception (%)*	46.6 ^a	33.6 ^b	35.0 ^x	17.3 ^y
Needles				
Needle inputs (g m ⁻² year ⁻¹)	160.9 ^a	183.7 ^a	104.5 ^x	118.5 ^x
Carbon:nitrogen†	96.2 ^a	82.3 ^b	99.9 ^x	64.2 ^y
Phosphorus (%)	0.11 ^a	0.11 ^a	0.12 ^x	0.17 ^y
Nitrogen (%)	0.48 ^a	0.57 ^b	0.55 ^x	0.79 ^y
Lignin (%)†	19.0 ^a	20.0 ^a	19.4 ^x	18.2 ^x
Lignin:nitrogen	40.5 ^a	35.5 ^b	38.0 ^x	22.5 ^y
Tannin (g kg ⁻¹)†	58.5 ^a	54.4 ^a	50.3 ^x	49.8 ^x
Roots				
Root inputs (g m ⁻² year ⁻¹)	68.9 ^a	47.4 ^a	65.0 ^x	53.0 ^x
Carbon:nitrogen‡	92.7 ^a	102.6 ^b	100.6 ^x	92.5 ^y
Phosphorus (%)‡	0.10 ^a	0.10 ^a	0.11 ^x	0.11 ^x
Nitrogen (%)‡	0.50 ^a	0.46 ^b	0.47 ^x	0.53 ^y
Lignin (%)‡	15.8 ^a	15.8 ^a	15.2 ^x	18.2 ^x
Lignin:nitrogen‡	31.6 ^a	34.6 ^a	33.0 ^x	35.0 ^x

*Data from Classen *et al.* (2005).

†Data from Chapman *et al.* (2003).

‡Data from five composites of initial sample in root decomposition bags.

Table 2. Number of trees used for needle and root collection, needle and root decomposition and needle and root nutrient inputs

	Tree group	Needle collection	Root collection*	Needle decomposition	Root decomposition	Needle inputs	Root inputs
Moth-susceptible	1	10					
	2		5				
	3			20	20	20	20
Moth-resistant	1	10					
	2		5				
	3			20	20	20	20
Scale-susceptible	1	10					
	2		7				
	3			20		20	
	4				20		
	5						20
Scale-resistant	1	10					
	2		7				
	3			20		20	
	4				20		
	5						20

*Roots were collect from a nearby site with similar soils, vegetation and rates of herbivory.

apparent needle N concentration by no more than 10%, probably considerably less given the loss of scales prior to needle chemical analysis (N. S. Cobb and G. W. Koch, unpublished data).

DECOMPOSITION AND NUTRIENT RELEASE

Because litter quality and soil microclimate interact to affect needle and root mass loss and nutrient release (Chapman *et al.* 2003; Classen *et al.* 2005), we initiated a 24-month reciprocal litter transplant study. Though other experimental designs exist, this approach allows us to disentangle the effects of microclimate and litter quality on nutrient processes (Robertson & Paul 2000; Appendix 1).

In May 2000, we randomly chose 20 moth-susceptible, 20 moth-resistant, 40 scale-susceptible and 40 scale-resistant trees at the site. Because our study focuses on individual trees, many with small crowns, we were unable to use the same trees for all measurements taken in this study without disturbing the tree crowns or root systems. To get around this potential problem, we increased the number of scale-trees examined as follows (and see Table 2). We placed both needle and root decomposition bags beneath moth-trees. However, owing to the small crowns of many scale-trees, we placed only needle or only root decomposition bags beneath scale trees. Thus, for each treatment, $n = 20$.

The needle decomposition bag design used in this study is described by Chapman *et al.* (2003). Briefly, the upper side of each 10 × 10 cm needle decomposition bag was constructed of 0.8 mm polyester mesh and the lower side (facing the ground) was constructed of 0.2 mm # 72191 polypropylene (Synthetic Industries, Atlanta, GA, USA). Bags were stitched together on three sides with polyester thread and closed with stainless steel staples.

Needle litter used to fill decomposition bags was collected in nylon mesh collectors suspended beneath the crowns of 10 trees in each of the four categories (moth and scale, resistant and susceptible) for 6 months in 1999. Dropped needles were collected bimonthly, air-dried and combined and homogenized within the moth-resistant, moth-susceptible, scale-resistant and scale-susceptible tree type. Decomposition bags placed beneath moth-susceptible and resistant trees contained approximately 2.5 g of needle litter, while bags placed beneath scale-susceptible and resistant trees contained approximately 1.25 g of needle litter. These amounts approximate the annual production of needle litter per unit ground area equal to that of the decomposition bags (0.01 m²). In total, we placed 160 needle decomposition bags in the field (4 treatments × 2 collection dates × 20 replicates).

Root decomposition bags were constructed as above except they were made entirely of 0.2 mm polypropylene. To avoid impact to the study trees, roots were collected from susceptible and resistant trees located at a nearby site with similar soil, vegetation and rates of herbivory

(Table 2). Roots were obtained using a hand blower, shovels and soil sieves from five moth-susceptible and five moth-resistant trees, and seven scale-susceptible and seven scale-resistant trees, briefly washed with DI water to remove cinders and hand sorted using visual characteristics to obtain live piñon roots (< 2 mm diameter). Live roots were used because of the difficulty of obtaining adequate quantities of recently senesced roots from these trees. Although live and senesced roots of piñon may differ chemically, root retranslocation of nutrients is generally very low (Nambiar & Fife 1991) and live roots are commonly used in decomposition experiments because of the difficulty of determining root senescence (e.g. Gholz *et al.* 2000). Hereafter, we refer to this collected root tissue as 'root litter'. Air-dried roots were cut into 5-cm lengths and placed into bags. Moth-susceptible and resistant root decomposition bags contained 1.25 g of air-dry roots while scale-susceptible and resistant root bags contained 0.625 g of air-dry roots. These amounts approximate annual fine root production per unit ground surface area equal to that of the root decomposition bag (0.01 m²). In total, we buried 160 root bags in the field (4 treatments × 2 collection dates × 20 replicates).

Decomposition bags were deployed in May (needles) and June (roots) of 2000. Bags of litter from moth-susceptible and resistant trees were paired and placed beneath crowns of moth susceptible and resistant trees, while bags of litter from scale-susceptible and resistant trees were paired and placed beneath crowns of scale-susceptible and resistant trees (Appendix 1). The co-location of bags containing litter from susceptible and resistant trees beneath both susceptible and resistant trees was designed to separate the effects of microclimate and chemical quality (Table 1; Appendix 1; Chapman *et al.* 2003). Needle litterbag pairs were secured on the soil surface midway between the trunk and the crown dripline at one of four randomly selected cardinal directions. Root litterbag pairs were inserted vertically into the top 15 cm of the mineral soil profile at the crown drip line. Pairs of litterbags were randomly selected and retrieved from beneath each tree after 12 and 24 months of decomposition.

We separately quantified needle and root litter chemical composition for the herbivore categories, and after 12 and 24 months of incubation. Analysis of these samples was similar to the sample analysis described above.

TOTAL N AND P INPUTS AND RELEASE

To determine how insect infestation impacts total needle and root litter inputs (production and nutrient content) and turnover, we used needle littertraps, root ingrowth bags, and the established decomposition experiment. For details on needle litterfall collection see Chapman *et al.* (2003).

Root production was assessed using root ingrowth bags (Hamzah *et al.* 1983) randomly located beneath susceptible and resistant crowns ($n = 20$). Differences

in root density due to tree age and size were accounted for by inserting three bags beneath moth-susceptible and resistant crowns and one bag beneath scale-susceptible and resistant crowns. Bags were installed by driving a bevelled polyvinyl chloride tube (8.89 cm diameter) to a depth of 30 cm at a location approximately 20 cm in from the canopy dripline. Soil was extracted with a sand auger and roots were removed by hand. A 50 cm long mesh (2 mm) bag was inserted into the tube, filled with the root-free soil, after which the tube was removed.

Roots grow slowly in this semi-arid environment, thus ingrowth bags were removed 1.5 years after insertion. Roots were extracted from cores using a hydropneumatic elutriator and stacked 1 mm and 500 μm sieves. Collected roots were identified as piñon live, piñon dead, juniper, or other species, by visual inspection with a dissecting scope. Collected piñon roots were oven-dried and analysed for N and P as described above.

Annual input of needle and root-litter N and P to the soil (g m^{-2}) for year one was calculated by multiplying the nutrient concentration in litterfall or new root (g g^{-1}) by the total litterfall or root production for that tree ($\text{g m}^{-2} \text{y}^{-1}$) (Hart *et al.* 1992). Net needle nutrient release at the end of 1 and 2 years was calculated by multiplying litterbag nutrient loss per g of litter by total litterfall ($\text{g m}^{-2} \text{year}^{-1}$). Net root nutrient release was calculated by multiplying the net change in N or P content of roots after 1 and 2 years of decomposition in decomposition bags by the annual root production determined from ingrowth bags. This calculation has three major assumptions: (i) on an annual basis, root production equals root mortality so that fine root biomass is in steady state; (ii) live root litter chemistry is similar to root litter chemistry at senescence (Nambiar & Fife 1991); and (iii) large interannual variations in litterfall and root production are likely to have a larger impact on these calculations than interannual variation in tissue chemistry (see Schuster *et al.* 2005).

DATA ANALYSIS

All statistical analyses were conducted using JMP 4 statistical software with alpha set *a priori* as $P < 0.05$ (SAS Institute, Pacific Grove, CA, USA, 2001). We analysed the effects of moths and scales on decomposition separately using a 2 (tree type: resistant or susceptible) \times 2 (litter type: from resistant or susceptible trees) \times 2 (time: year 1 or year 2) full factorial fixed effect ANOVA within each herbivore type. When there was a significant time effect, we used a separate 2 \times 2 model within each year to understand better the statistical interactions between factors. ANOVAs were conducted separately for needle and root mass loss and N and P release (% remaining). Data that violated the normality assumption of ANOVA and proportional data were transformed: moth and scale needle and scale root mass remaining were arcsine square root transformed,

and moth needle N remaining and root N and P remaining were log-transformed. One concern might be that this design is pseudoreplicated because we placed litter from resistant and susceptible trees beneath the same tree type. However, such a design allows us to explicitly decouple the effects of microclimate and litter quality on decomposition and nutrient release (Aaron M. Ellison, personal communication). To ensure that we were not biasing our interpretation of the patterns we detected (see Results), we explored our data using two alternative approaches. First, to take advantage of the fact that we paired litter types beneath tree types, we examined the contrast between litter types under each tree and then used 2 \times 2 ANOVA models with tree type and year on the contrast value. Secondly, we used another series of 2 \times 2 ANOVA models with tree type and year on the average of the litter types beneath each tree. The results from these analyses were qualitatively similar to those presented here. Therefore, to make our results comparable with previous methods and studies in both this system (e.g. Chapman *et al.* 2003; Langley *et al.* 2006) and others (e.g. Hart *et al.* 1992; Robertson & Paul 2000; Balsler & Firestone 2005; Madritch *et al.* 2007), we present the quantitative results from the 2 (tree type) \times 2 (litter type) \times 2 (year) described above.

To explore relationships between herbivore-driven changes in crown architecture and decomposition, LAI data collected (published in Classen *et al.* 2005) were regressed against mass remaining. A stepwise multiple regression analysis on several microclimate parameters found that yearly average maximum soil temperature (at 5 cm) and yearly cumulative crown throughfall were the best predictors of needle litter mass loss after 2 years. Yearly average soil maximum temperature (at 5 cm) and cumulative crown throughfall were not correlated with each other ($R^2 = 0.01$, $P = 0.69$) and thus could be used as independent variables in a stepwise multiple regression.

Differences between herbivore-susceptible and resistant root and needle litter N and P inputs in year one and release after year one and year two were tested using ANOVA. Native conditions (susceptible litter located beneath a susceptible tree and resistant litter located beneath a resistant tree) were tested with tree type (susceptible, resistant) as the main effect for release data. Non-native comparisons were not included in this manuscript because they do not realistically represent how susceptibility will shape this ecosystem in which trees are isolated on the landscape. All data are shown as non-transformed mean values in figures and tables. Where appropriate, data are shown on an ash-free, oven-dry basis.

Results

CHEMICAL QUALITY

Moth-susceptible needle litter ($F_{1,37} = 5.26$, $P = 0.03$) and scale-susceptible needle litter ($F_{1,36} = 50.86$, $P < 0.01$)

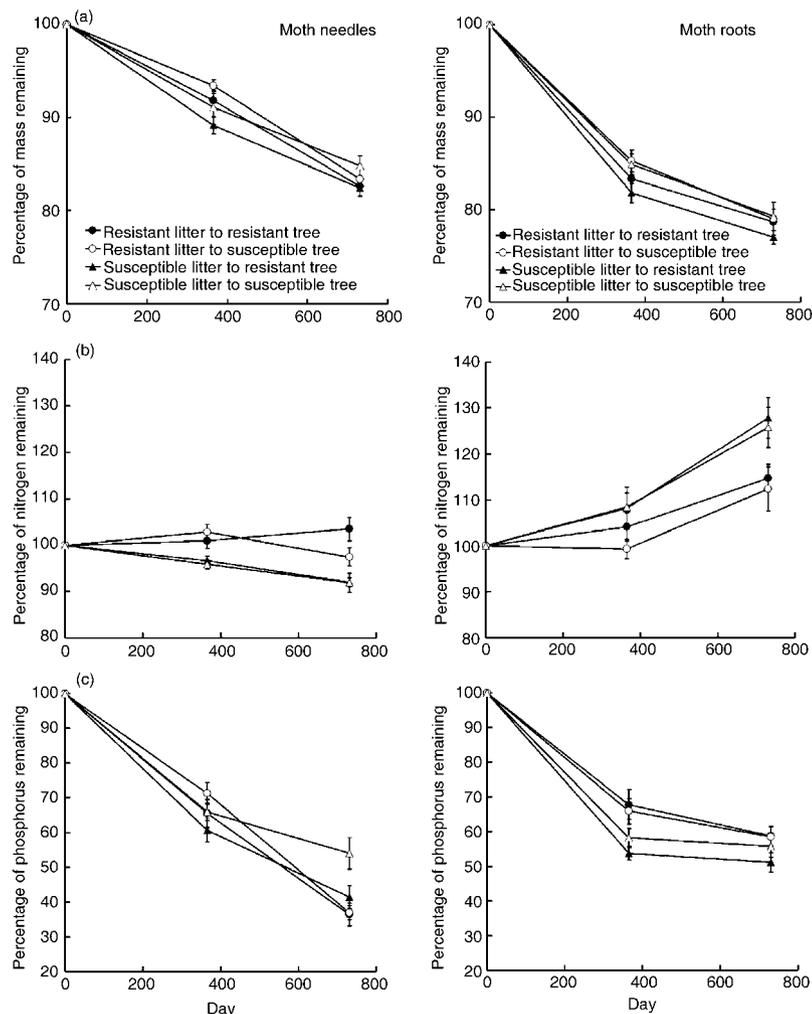


Fig. 1. Moth susceptibility and resistance effects on needle and root: (a) percentage of mass remaining, (b) percentage of nitrogen remaining, and (c) percentage of phosphorus remaining over 2 years. Treatments include trees that were moth-susceptible and resistant to herbivory. Vertical bars are ± 1 SE.

had higher [N] and moth-susceptible ($F_{1,37} = 5.84$, $P = 0.02$) and scale-susceptible ($F_{1,36} = 59.52$, $P < 0.01$) needle litter had lower C:N-values than did moth-resistant and scale-resistant needle litter. Scale-susceptible needle litter had higher P concentration ($F_{1,36} = 50.86$, $P = 0.01$) and lower lignin:N-values than did scale-resistant needle litter ($F_{1,14} = 25.59$, $P < 0.01$; Table 1). Lignin and condensed tannin concentrations were similar between moth-susceptible and moth-resistant and scale-susceptible and scale-resistant needle litter (Table 1).

Herbivore susceptibility also influenced root litter chemistry. Moth-susceptible root litter had lower [N] ($F_{1,6} = 45.63$, $P < 0.01$) and higher C:N ($F_{1,6} = 92.96$, $P < 0.01$) than moth-resistant root litter. Scale-susceptible root litter had higher [N] ($F_{1,6} = 226.71$, $P < 0.01$) and lower C:N ($F_{1,6} = 33.20$, $P < 0.01$; Table 1) than scale-resistant root litter. Both moth-susceptible and moth-resistant and scale-susceptible and scale-resistant root litter [P], % lignin, and lignin:N-values were not significantly different (Table 1).

DECOMPOSITION AND NUTRIENT RELEASE

Mass loss from moth-susceptible and moth-resistant litter was largely driven by changes in microclimate and not by the effect of moth-susceptibility or resistance on litter chemistry. There was a time \times litter type interaction ($F_{1,143} = 58.55$, $P < 0.01$; Fig. 1, Table 3) where, when doing separate ANOVAs for 2001 and for 2002, moth-susceptible needle litter lost more mass than did moth-resistant litter in 2001 ($F_{1,76} = 4.58$, $P = 0.04$; Fig. 1) and litter located beneath moth-resistant trees lost more mass than litter located beneath moth-susceptible trees ($F_{1,76} = 4.37$, $P = 0.04$). There was no effect of litter type on location or mass loss in 2002. Root litter located beneath moth-resistant crowns lost more mass than did litter located beneath moth-susceptible crowns ($F_{1,151} = 145.92$, $P = 0.02$; Fig. 1, Table 3) and there were no interactions. There was a significant effect of time on moth-susceptible and moth-resistant needle litter decomposition ($F_{1,151} = 1121.03$, $P < 0.01$).

Table 3. Probability values and *F* statistics generated using a three-way ANOVA of herbivore-susceptible and resistant needle and root: (a) mass remaining, (b) nitrogen remaining, and (c) phosphorus remaining over a 2-year period. Litter type refers to the source of roots or needles from either resistant or susceptible trees. Location refers to where the roots were decomposed (beneath resistant or susceptible crowns). Bold numbers indicate significant differences ($P \leq 0.05$). *F*-values are in parenthesis located beside *P*-values. 'ND' stands for no detectable difference. Non-significant interactions were not included in this table

	d.f.	Needles		Roots	
		Moth	Scale	Moth	Scale
(a) Mass remaining (%)					
Model	7	<< 0.01 (20.03)	<< 0.01 (20.65)	<< 0.01 (7.93)	0.17 (1.52)
Effect					
Time	1	<< 0.01 (125.98)	<< 0.01 (139.11)	<< 0.01 (46.38)	ND
Litter type	1	0.17 (1.89)	0.82 (0.05)	0.30 (1.08)	ND
Location	1	< 0.01 (6.90)	0.91 (0.01)	0.02 (6.04)	ND
Time × Litter type	1	0.04 (4.26)	0.34 (0.91)	0.88 (0.02)	ND
(b) Nitrogen remaining (%)					
Model	7	<< 0.01 (11.02)	<< 0.01 (4.06)	<< 0.01 (7.05)	<< 0.01 (3.14)
Effect					
Time	1	0.27 (1.20)	0.27 (1.21)	<< 0.01 (32.28)	0.08 (3.05)
Litter type	1	<< 0.01 (11.55)	<< 0.01 (7.06)	<< 0.01 (14.07)	<< 0.01 (11.68)
Location	1	0.67 (0.18)	0.50 (0.46)	0.32 (0.98)	0.05 (3.88)
Time × Litter type	1	<< 0.01 (58.55)	<< 0.01 (11.51)	0.27 (1.21)	0.27 (1.22)
(c) Phosphorus remaining (%)					
Model	7	<< 0.01 (17.05)	<< 0.01 (18.38)	<< 0.01 (3.46)	<< 0.01 (6.47)
Effect					
Time	1	<< 0.01 (98.56)	<< 0.01 (122.38)	<< 0.01 (6.98)	<< 0.01 (19.22)
Litter type	1	0.21 (1.57)	0.77 (0.12)	<< 0.01 (13.67)	<< 0.01 (19.89)
Location	1	0.01 (6.43)	0.06 (3.59)	0.39 (0.72)	0.77 (0.08)
Time × Litter type	1	<< 0.01 (11.37)	0.45 (0.57)	0.32 (0.98)	0.50 (0.46)
Time × Location	1	0.83 (0.05)	0.64 (0.22)	0.84 (0.04)	< 0.05 (3.94)

*Interactions that are not presented in this table were not significant.

Similar to moth herbivory, there was no detectable difference between scale-susceptible and scale-resistant needle or root litter mass loss (Fig. 2, Table 3), even with a 30% increase in initial scale-susceptible needle litter N concentration relative to scale-resistant litter (Table 1). There was, however, a significant effect of time on scale-susceptible and scale-resistant needle litter decomposition ($F_{1,152} = 139.11$, $P < 0.01$; Fig. 2), where needles in year two lost more mass than needles in year one.

Exploring these findings using regression analysis across all treatments demonstrated that tree architecture and microclimate explain much of the variation in needle litter mass loss. Leaf area index alone predicted 30% of the variation ($R^2 = 0.30$, $P < 0.01$; Fig. 3), while a multiple regression containing yearly average maximum soil temperature (5 cm) and yearly cumulative crown throughfall explained 69% of the variation in needle mass loss after 2 years ($R^2 = 0.69$, $P < 0.01$; Fig. 3).

NITROGEN RELEASE

While location generally influenced moth-susceptible and resistant litter mass loss, litter type (i.e. from resistant or susceptible trees) often influenced N dynamics. Moth-susceptible needle litter mineralized N, while

moth-resistant needle litter immobilized N. While these patterns were significant ($F_{1,143} = 11.55$, $P < 0.01$; Fig. 1), there was a small (9%) difference in N immobilization between moth-susceptible and resistant needle litter after 2 years and there was a significant time × litter type interaction ($F_{1,143} = 58.55$, $P < 0.01$; Fig. 1). Root litter showed an opposite response to moth-susceptibility, and the magnitude of the responses was slightly larger. Moth-susceptible root litter immobilized 11% more N than moth-resistant root litter ($F_{1,151} = 14.07$, $P < 0.01$; Fig. 1, Table 3). There was also a significant effect of time on moth-susceptible and resistant root litter decomposition ($F_{1,151} = 32.27$, $P < 0.01$; Fig. 1).

Scale-susceptibility also had a significant impact on needle litter N dynamics ($F_{1,149} = 7.06$, $P < 0.01$, Fig. 2, Table 3). There was a time × litter type interaction ($F_{1,149} = 11.51$, $P < 0.01$; Table 3, Fig. 2), where, when doing separate ANOVAs for year one and year two, scale-resistant needles lost more N than scale-susceptible needles in year one ($F_{1,76} = 12.46$, $P < 0.01$; Fig. 2), but this effect was not detectable in year two. Across all treatments, root litter immobilized N, and scale-susceptible root litter immobilized 11% more N than did scale-resistant root litter ($F_{1,144} = 11.68$, $P < 0.01$; Fig. 2, Table 3).

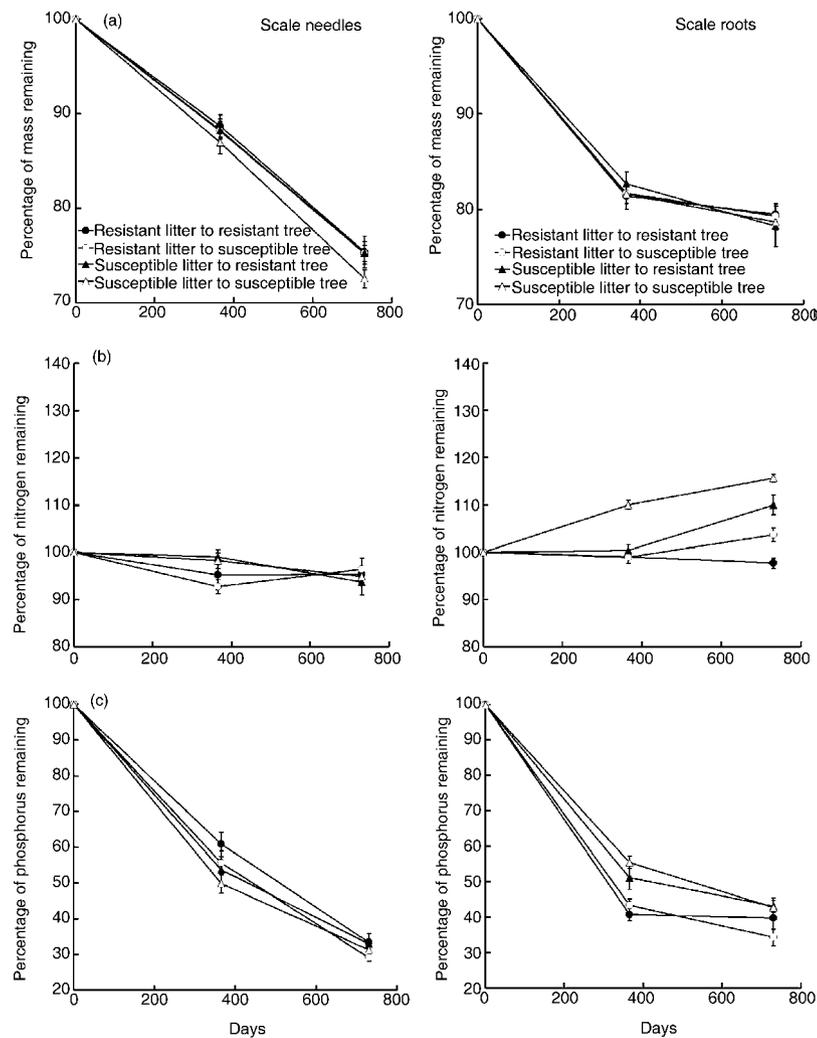


Fig. 2. Scale susceptibility and resistance effects on needle and root: (a) percentage of mass remaining, (b) percentage of nitrogen remaining, and (c) percentage of phosphorus remaining over 750 days. Treatments include trees that were scale-susceptible and resistant to herbivory. Vertical bars are ± 1 SE.

PHOSPHORUS RELEASE

Moth susceptibility and resistance had mixed effects on net P release from both needle and root litter. There was a time \times litter type interaction ($F_{1,143} = 11.37$, $P < 0.01$; Fig. 1, Table 3); moth-susceptible needle litter released less P than moth-resistant needle litter in year two ($F_{1,67} = 10.33$, $P < 0.01$; Fig. 1), but not in year one. Conversely, moth-susceptible root litter released more P than did moth-resistant root litter, independent of decomposition location and year ($F_{1,151} = 13.66$, $P < 0.01$; Fig. 1, Table 3).

Scale susceptibility and resistance also had significant effects on P release from litter. While there was no effect of scale-susceptibility or scale resistance on P release from needle litter, there was a significant effect of time on scale-susceptible and resistant needle litter P release ($F_{1,149} = 122.38$, $P < 0.01$; Fig. 2, Table 3). Similar to moth-susceptibility, scale root litter type, but not location, drove differences in root P release. There was a time \times location interaction ($F_{1,144} = 3.94$, $P < 0.05$; Fig. 2, Table 3), where scale-resistant litter beneath

resistant trees was indistinguishable from susceptible litter in year two. When doing separate ANOVAs for year one and year two, scale-susceptible root litter released less P than did scale-resistant root litter in both year one ($F_{1,68} = 15.08$, $P < 0.01$) and year two ($F_{1,76} = 6.57$, $P = 0.01$). Across treatments, there was a net release of P, and this increased from year one to year two.

TOTAL NITROGEN & PHOSPHORUS INPUTS

With one exception, moth and scale-susceptibility and resistance did not affect total nutrient input (Table 4). Scale-susceptibility increased needle litter N inputs by 42% relative to scale-resistant needle litter inputs ($F_{1,26} = 4.97$, $P = 0.03$; Table 4), but did not affect inputs of root N and P and needle P.

TOTAL NITROGEN RELEASE

In summary, moth-susceptible needle litter released N, while moth-resistant litter immobilized N after 1 year ($F_{1,34} = 4.82$, $P = 0.04$; Table 4), but moth-susceptibility

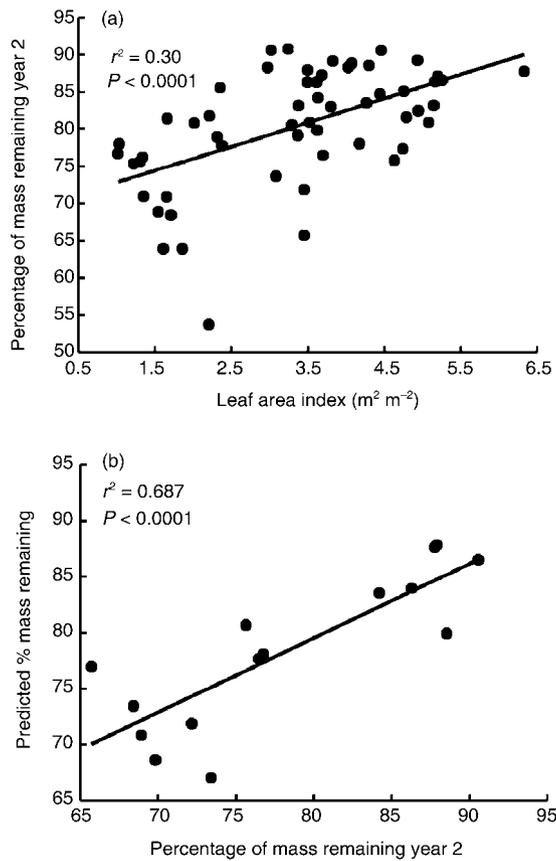


Fig. 3. (a) Percentage mass remaining is significantly correlated with leaf area index (LAI) after 2 years of needle litter decomposition. Points represent individual trees. (b) A regression model that combines annual cumulative crown throughfall with yearly average maximum soil temperature at 5 cm significantly predicts needle litter decomposition after 2 years.

had no effect on needle litter N release at the end of 2 years. Moth-susceptibility also had no effect on root litter N-release.

Relative to scale-resistant trees, scale-susceptible trees had 14% higher needle litter N immobilization after year one ($F_{1,26} = 4.14$, $P = 0.05$; Table 4), while after 2 years, scale-susceptible litter had 136% higher N release relative to scale-resistant litter ($F_{1,26} = 4.74$, $P = 0.04$; Table 4). These results reflect the relatively large (42%) increase in litter N inputs beneath scale-susceptible trees relative to resistant trees. Scale-susceptibility had no effect on root litter N release after year one, but root litter from scale-susceptible trees immobilized 44% more N than did root litter from scale-resistant trees at the end of 2 years ($F_{1,26} = 3.96$, $P = 0.05$; Table 4).

TOTAL PHOSPHORUS RELEASE

Moth-susceptibility and moth-resistance had no measurable impact on needle or root litter P release. However, P release was approximately 2.5 times greater from scale-susceptible needle litter than from scale-resistant needle litter after 1 ($F_{1,26} = 6.74$, $P = 0.02$) and 2 years of decomposition ($F_{1,25} = 8.15$, $P < 0.01$; Table 4). In contrast, P release was up to 48% lower from scale-susceptible root litter than from scale-resistant root litter after year one ($F_{1,38} = 15.43$, $P < 0.01$) and after year two ($F_{1,38} = 103.12$, $P < 0.01$; Table 4).

Discussion

Our research supports five major conclusions. (i) Moth-susceptibility effects on needle and root litter mass loss

Table 4. Herbivore susceptibility and resistance effects on estimated rates of annual net nitrogen (N) and phosphorus (P) release after years one and two and total N and P input for year one. Standard errors are shown in parentheses beside means. Within rows, contrasting letters denote significant differences using a Tukey HSD test ($P \leq 0.05$) within scale and moth trees. Negative values denote net immobilization.

Time	Needles		Roots	
	Resistant	Susceptible	Resistant	Susceptible
Moth				
Year 1				
Total N input (mg m ⁻² y ⁻¹)	895.5 ^a (132.7)	1194.9 ^a (137.0)	207.8 ^a (39.0)	184.8 ^a (86.6)
Net N release (mg m ⁻² y ⁻¹)	-10.9 ^a (18.4)	44.2 ^b (17.0)	-24.5 ^a (17.6)	-31.2 ^a (12.7)
Total P input (mg m ⁻² y ⁻¹)	192.4 ^a (35.0)	223.5 ^a (29.3)	48.9 ^a (10.0)	43.2 ^a (20.7)
Net P release (mg m ⁻² y ⁻¹)	80.75 ^a (12.0)	107.4 ^a (16.6)	32.1 ^a (4.4)	34.7 ^a (2.3)
Year 2				
Net N release (mg m ⁻² y ⁻¹)	23.4 ^a (53.3)	76.1 ^a (26.5)	-86.4 ^a (17.9)	-96.1 ^a (16.0)
Net P release (mg m ⁻² y ⁻¹)	158.75 ^a (23.8)	154.4 ^a (21.2)	41.3 ^a (2.6)	6.9 ^a (2.5)
Scale				
Year 1				
Total N input (mg m ⁻² y ⁻¹)	554.3 ^a (96.6)	951.5 ^b (143.0)	340.1 ^x (79.6)	304.6 ^x (133.8)
Net N release (mg m ⁻² y ⁻¹)	22.5 ^x (17.5)	-19.77 ^y (12.0)	-21.3 ^x (4.6)	-23.5 ^x (6.2)
Total P input (mg m ⁻² y ⁻¹)	134.6 ^x (36.5)	197.0 ^x (29.2)	81.3 ^x (20.2)	69.1 ^x (32.0)
Net P release (mg m ⁻² y ⁻¹)	59.1 ^x (12.3)	125.1 ^y (21.1)	37.6 ^x (1.2)	22.6 ^y (0.9)
Year 2				
Net N release (mg m ⁻² y ⁻¹)	-36.3 ^x (33.7)	99.9 ^y (50.3)	-20.7 ^x (1.7)	-36.7 ^y (7.8)
Net P release (mg m ⁻² y ⁻¹)	83.8 ^x (17.3)	175.8 ^y (26.6)	38.1 ^x (2.0)	28.6 ^y (1.4)

are largely driven by variation in microclimate associated with herbivore impacts and not by differences in litter quality. (ii) Herbivore susceptibility effects on litter N release were driven largely by litter chemical quality and not by decomposition location (i.e. microclimate). In contrast, P release from needle litter was influenced by location (microclimate), while P release from roots was influenced by litter quality. In general, root decomposition in the soil profile appears to be less affected by the microclimate extremes at the soil surface, enabling differences in herbivore-caused variation in root chemical composition to be a stronger driver of nutrient dynamics. (iii) Herbivore-susceptibility exerted a large influence on net nutrient release, but the direction of herbivore influence was time dependent. (iv) Taken together, these results demonstrate that plant genetic susceptibility or resistance to herbivores can have different and often contrasting effects on needle and root litter mass loss and nutrient release. (v) Finally, our findings show significant plant genetic effects on above- and below-ground carbon and nutrient fluxes. If such effects are common, as we suspect, then it is important for ecosystem scientists to design experiments to explicitly incorporate a genetic perspective into their studies, particularly those focused on individual species of dominant plants. If changes in climate differentially affect the survival of resistant and susceptible trees or different plant genotypes in an ecosystem, there may be large impacts on whole ecosystem nutrient cycling.

INFLUENCE OF SUSCEPTIBILITY AND RESISTANCE ON NEEDLE AND ROOT DECOMPOSITION

Our results demonstrate that the effects of plant susceptibility and resistance to herbivores alter above- and below-ground litter nutrient dynamics, but that the best predictor for litter mass loss, across roots and needles, is microclimate, which varied with litter location in this study. These results are important because litter chemical quality has been shown to drive mass loss in a variety of ecosystems (Melillo *et al.* 1982; Parton *et al.* 2007) and is often cited as the most important driver of decomposition at the local scale (Köchy & Wilson 1997; Hobbie *et al.* 2006). Herbivores in this ecosystem have a large impact on needle litter quality, increasing needle litter N content up to 30%, and herbivore driven differences in mass loss rates after 1 year were found to be driven by differences in needle litter quality (Chapman *et al.* 2003). Interestingly, even with these large differences in herbivore-susceptible and resistant needle litter, needle litter nutrient content had little impact on mass remaining. In fact, scale susceptibility, which altered needle litter quality the most, had no impact on mass and phosphorus remaining after 2 years of decomposition. However, litter quality significantly altered both needle and root litter N dynamics and root litter P dynamics. These data suggest the impact of

herbivory on tree architecture, and thus, microclimate, appears to be the primary mechanism by which genetic susceptibility to herbivores alters needle litter mass loss and phosphorus dynamics in this semi-arid woodland. In contrast, the effect of herbivore susceptibility on litter chemical quality consistently influenced below-ground (root) nutrient dynamics. It is likely that subsurface soil moisture and temperature are more buffered and less variable among trees of different herbivory levels than the soil surface, thus allowing root chemical quality to dictate decomposition (McTiernan *et al.* 2003).

That the influence of herbivore susceptibility on tree architecture and consequent variation in soil surface microclimate plays a dominant role in this system is supported by the significant positive correlation between needle litter mass dynamics and LAI (Fig. 3). Our study design did not allow separation of LAI influences associated with variation in crown penetration of precipitation vs. UV light, the latter being known as an important driver of decomposition in arid ecosystems (Austin & Vivanco 2006). However, scale-susceptible trees (Fig. 3) have the lowest LAI, and their sparse crowns may intensify decomposition by increasing UV penetration relative to the crowns of moth trees. The level of UV light is unlikely to affect the below-ground decomposition of roots, and this may be the basis for the decoupling of the drivers of root and needle litter decomposition in this system. The different dynamics of root and needle litter decomposition are consistent with this interpretation. During the second year, root decomposition rates declined, whereas needle litter decomposition remained constant. During the second year of the experiment one of the most severe droughts in Arizona history occurred; thus, the photo-degradation of needle litter would continue, while the biotic degradation of roots may have slowed due to severe water limitation (Moorhead & Reynolds 1991; Moorhead & Reynolds 1991; Kemp *et al.* 2003).

An alternative interpretation may be that herbivore susceptibility effects on root litter nutrient dynamics are more consistently linked to litter chemical quality even though roots have smaller differences in litter quality than needles (Table 1). Previous research in arid environments established that root decomposition is often determined by chemical composition, whereas leaf decomposition is determined by both microclimate and chemical composition (Silver & Miya 2001; Moretto & Distel 2003). These soils are young, nutrient poor, and depauperate of organic matter; inputs of litter to the below-ground environment may thus represent an influx of limiting substrates. Microbial decomposition of root litter may therefore be governed by nutrient availability, as determined by herbivore alterations in litter chemical quality. Conversely, the influx of needle litter occurs in the more developed portion of the soil profile in this environment, where nutrients are somewhat less limiting and therefore chemical quality may be less important. Together these results indicate

that decomposition may not be regulated by consistent or 'traditional' chemical quality mechanisms (i.e. C:N, lignin:N), and this may be especially true in arid ecosystems (e.g. Schaefer *et al.* 1985; Whitford *et al.* 1986; Silver & Miya 2001; Austin & Vivanco 2006; Parton *et al.* 2007).

HERBIVORE SUSCEPTIBILITY ALTERS NET NUTRIENT RELEASE: MECHANISMS AND IMPLICATIONS

Herbivore susceptibility in this system exerts a large influence on nutrient cycling, and the direction (immobilization vs. release) often changes over time. For example, after 1 year, scale susceptibility caused net N immobilization; whereas net N mineralization occurred beneath resistant trees. The patterns were opposite the following year where scale susceptibility caused net N release and resistant tree litter showed net immobilization. This pattern could be driven by a faster progression through the various stages of N dynamics (N release due to leaching, followed by a period of immobilization, and eventual N release) due to higher N concentration in scale-susceptible litter. Unlike N, P dynamics were similar in both years, but differed between root and needle release. Scale susceptibility increased the net release of P from needle litter into the soil after both 1 and 2 years of decomposition; whereas it decreased the release of P from root litter. In these relatively P-rich ecosystems, it is possible that active microbial communities in the more moist litter beneath scale trees processed the litter faster, leading to a larger net P release. Regardless of the mechanism, scale susceptibility effected large changes on N and P cycling in this nutrient-limited ecosystem, potentially altering the future productivity of moth and scale trees.

Understanding the complexity of above- and below-ground interactions is an emerging challenge (Wardle *et al.* 2004). In addition, with few exceptions, researchers have overlooked root decomposition as an important regulator of nutrient cycling in ecosystem models (Meentemeyer 1978; Aerts 1997), even though a large portion of annual NPP is partitioned below-ground (Vogt *et al.* 1986). Our finding that root and needle litter decomposition and nutrient dynamics are often controlled by contrasting factors (here, microclimate and litter quality) supports previous research (Ostertag & Hobbie 1999). Further, our investigation of herbivory in the context of above- and below-ground decomposition advances our understanding of biotic controls on litter dynamics and documents that a single factor can affect above- and below-ground responses differently (Ostertag & Hobbie 1999). The present study suggests that important components of nutrient cycling may be incorrectly estimated by drawing conclusions only from leaf litter mass loss over short time periods, especially when herbivory is present. Although some studies have attempted to address the genetic components of ecosystem processes (e.g. Schweitzer *et al.* 2004; Madritch *et al.*

2006), our findings argue that a genetics approach could play an important mechanistic role and provide new tools to explore fundamental ecosystem processes. For example, common gardens composed of high and low lignin genotypes, drought tolerant and intolerant genotypes, and/or high and low productivity genotypes, and coupled with mixed water and nutrient treatments, could provide invaluable findings on the genetic × environment interactions that affect major ecosystem processes.

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Appendix 1

Reciprocal transplant experimental design. Litterbags with different litter qualities were paired and placed beneath each tree-type

Litter quality	Tree type Susceptible	Resistant
Susceptible	Susceptible control ($n = 20$)	Interaction between canopy architecture effects on microclimate and litter quality ($n = 20$)
Resistant	Interaction between canopy architecture effects on microclimate and litter quality ($n = 20$)	Resistant control ($n = 20$)