Biogeochemistry **64:** 129–148, 2003.

Polyphenols in litter from tropical montane forests across a wide range in soil fertility

S. HÄTTENSCHWILER[1,3,*], A.E. HAGERMAN[2] and P.M. VITOUSEK[1]

*[1]Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA; [2]Department of Chemistry and Biochemistry, Miami University, Oxford, OH 45056, USA; [3]Current address: Institute of Botany, University of Basel, Schönbeinstr. 6, 4056 Basel, Switzerland; *Author for correspondence (e-mail: stephan.haettenschwiler@unibas.ch; fax +41 61-267-3504)*

Received 1 October 2001; accepted in revised form 24 May 2002

Key words: Condensed tannins, Fertilization, Genotypic and phenotypic variation, Hawai'i, Leaf and fine root litter, Nitrogen, Nutrient availability, Phenolics, Phosphorus, Proanthocyanidins, Soil chronosequence

Abstract. In nutrient-poor ecosystems high polyphenol concentrations in plant litter have been proposed to influence soil nutrient availability in benefit of the plants. We addressed the question whether litter polyphenol concentrations vary across a soil chronosequence of almost identical geology, climate and plant species composition, but of a wide range in nitrogen (N) and phosphorus (P) availability in the Hawaiian Islands. Concentrations of total phenolics (TPh) and proanthocyanidins (PA) in leaf litter of the dominant tree species *Metrosideros polymorpha* were higher at the oldest, P-limited site compared to the youngest, N-limited site, with intermediate values at the two relatively fertile sites co-limited by N and P. Polyphenol concentrations in fine root litter differed considerably from those observed in leaf litter and varied differently across the soil age gradient. Long-term fertilization did not significantly alter polyphenol concentrations in *Metrosideros* litter at either site. Moreover, green leaves and leaf litter of *Metrosideros* showed similar relative differences among sites when compared between natural populations and plants from the same populations but grown in a common garden. These results suggest that polyphenol concentrations inherently vary among populations of the dominant tree species in Hawaiian montane forests possibly indicating an adaptation to ecosystem properties such as substrate age related differences in soil fertility. The combined above- and below-ground input rate of TPh ranged from 62.4 to 170.8 g/m^2/yr and was significantly higher at the P-limited than at the N-limited site. Root-derived polyphenols contributed a much higher absolute and relative amount of phenolic input at the N-limited than at the P-limited site. The differences in amount, quality, and pathways of input might suggest specific interactions with soil processes and nutrient cycling among the Hawaiian rainforests studied here.

Introduction

Polyphenols are the predominant class of plant secondary metabolites, occurring widely and often in large quantities in plant tissues (Bate-Smith 1962; Swain 1979; Haslam 1989; Harborne 1997). The observed high variation of polyphenol concentration among and within species has been discussed in relation to their function as carbon-based defense compounds against herbivores and pathogens (Coley et al. 1985; Lamb et al. 1989; Herms and Mattson 1992; Hartley and Jones 1997; Karban and Baldwin 1997). Better-defended tissues in living plants may result in slower decomposition rates after senescence, as was suggested by Grime et al. (1996) in a comparative analysis. Damage to living leaves of poplar trees (*Populus deltoides*) caused a higher concentration of polyphenols in leaf litter of these plants, and was followed by slower decomposition (Findlay et al. 1996). In addition, high polyphenol concentrations in plant litter may be an adaptive response to infertile soils (Northup et al. (1995b, 1998)) regardless of any functions in living tissues. A number of experiments indicate that plant-derived polyphenols can affect a variety of soil processes (see reviews by Horner et al. (1988) and Kuiters (1990), Hättenschwiler and Vitousek (2000)), some of which could increase nutrient availability to plants.

Large responses of polyphenol concentrations to differences in soil N availability are well documented for green leaves of a variety of plant species (Gershenzon 1984; Bryant et al. 1987; Ruohomäki et al. 1996; Haukioja et al. 1998; Hättenschwiler and Schafellner 1999). These observations are generally in line with the theoretical predictions of a higher phenolic concentration at lower levels of nutrient availability, based on either the carbon nutrient balance hypothesis (CNB, Bryant et al. (1983)) or the more recent protein competition model (PCM, Jones and Hartley (1999)) of phenolic allocation. The variability of polyphenol concentration in litter is much less studied, but consistent with theory and data on green leaves, Northup et al. (1995a) found increasing tannin concentration in pine needle litter along a natural soil fertility gradient of decreasing N availability. Ecosystem-level interpretations of the observed changes in polyphenol concentrations remains difficult, however, due in part to limited data on above- and particularly below-ground inputs of plant-produced polyphenols into the soil. Concentrations of polyphenols within a single plant species need not correlate with the total amount of polyphenols produced and transferred to the soil per unit ground area, either within that species or at the community level. Moreover, we do not know of any attempt to separate phenotypic variation in response to changing nutrient availability from genotypic variation in response to site-specific differences in soil fertility, within the same plant species. Such distinction, however, is important in an assessment of selection for polyphenol-rich genotypes on infertile soils.

The evaluation of below-ground, root-derived polyphenols may be especially important to understanding how polyphenols influence the availability and uptake of nutrients by plants, because polyphenols produced by roots are more widely distributed within the soil, and are more closely related to processes in the rhizosphere, than polyphenols released from litter deposited on the soil surface. Limited evidence suggests that the phenolic composition of fine roots can differ greatly from that of leaves within the same plant species (Gallet and Lebreton 1995).

In this study we address how polyphenol concentrations in leaf and root litter of Hawaiian tropical montane forests vary across a substrate age gradient that includes sites of a wide range in nitrogen and phosphorus availability. This gradient enabled us to identify patterns of litter polyphenol concentrations in natural populations of the same plant species in response to differences in soil fertility. In addition, responses of litter polyphenol concentrations to long-term fertilization were determined within three of the sites. Finally, we evaluated phenotypic vs. genotypic responses using plants originating from the different study sites, but growing in the same environment in a common garden.

Material and methods

Study sites

Four montane rainforest sites on the Hawaiian islands from the chronosequence described by Crews et al. (1995) were included in this study. Three sites (Thurston, Laupahoehoe, and Kohala) are located on the island of Hawaii and one site is located on the island of Kauai (Kokee). Soils at all sites derived from volcanic tephra ranging in age from 300 years at the youngest site (Thurston) to 4.1 million years at the oldest site (Kokee, Table 1). All four sites are characterized by a mean annual precipitation near 2500 mm (Giambelluca et al. 1986) and an altitude near 1200 m, corresponding to a mean annual temperature of 16 °C (Crews et al. 1995). Each site is covered by similar native forest vegetation (Kitayama and Mueller-Dombois 1995) that is dominated by the tree species *Metrosideros polymorpha*; none appear to have been cleared by humans. *Metrosideros* constitutes more than 80% of the total community basal area at each site (Table 1). The most common co-occurring canopy trees are *Cheirodendron trigynum* and *Ilex anomala*, while *Coprosma* spp., *Myrsine* spp., *Cibotium* spp. and *Vaccinium calycinum* are abundant in the sub-canopy. *Cibotium* tree ferns are abundant at Thurston, and decrease in density with increasing substrate age. *Cibotium* is rarely found at Kokee, but other ferns, particularly *Elaphoglossum* spp., dominate the understory.

Total basal area and net primary production (above- and below-ground NPP combined) are similar at all four sites (Table 1), even though soil nutrient availability and foliar nutrient concentrations differ markedly (Crews et al. 1995; Vitousek et al. 1995). Nutrient limitation to net primary production has been identified by long-term factorial fertilization experiments at three of the four sites. These demonstrated that tree growth and above-ground net primary production (ANPP) is limited by N alone at the youngest site (Thurston), by P alone at the oldest site (Kokee), and is co-limited by N and P at the most fertile site of intermediate age (Laupahoehoe; see Vitousek et al. (1993) and Herbert and Fownes (1995), Vitousek and Farrington (1997)).

Plant sampling

Above-ground plant litter was collected at all four sites and within each of four plots of the fertilization treatments (control, N, P, and N+P) in July 1999, December 1999, and May 2000. Six (Laupahoehoe) or 10 (all other sites) litter traps covering approximately 1% of the total plot area were randomly distributed within each plot, and litter was collected three days later. If not enough litter accumulated over three days, the litter traps remained in place for another three days. The relatively

Table 1. Soil and forest stand characteristics of the four study sites along the natural fertility gradient in the Hawaiian Islands.

		Thurston	Laupahoehoe	Kohala	Kokee
Soil age (10^3 yr)[1]		0.3	20	150	4100
Soil pH (in water)[1]		5.0	3.6	4.1	4.0
Total soil C (kg/m^2)[1]		15.3	32.4	33.6	24.1
Total soil N (kg/m^2)[1]		0.98	1.56	1.46	1.13
In situ resin extractable nutrients (mg/bag/d)[1]	NH_4-N	3.01	8.12	4.90	4.12
	NO_3-N	0.22	4.25	0.31	10.29
	P	0.20	1.21	2.19	0.41
Foliar nutrients in *Metrosideros* (% d.m.)[2]	N	0.87	1.42	1.14	0.86
	P	0.060	0.101	0.113	0.061
Litter nutrients in *Metrosideros* (% d.m.)[1]	N	0.40	0.80	0.74	0.37
	P	0.026	0.053	0.054	0.022
Basal area (m^2/ha)[3]		36.7	32.0	36.6	36.8
Metrosideros basal area(% of total)[3]		81	83	83	88
Total NPP (g/m^2/yr)[3]		1577	1604	1800	1514

[1] Data from Crews et al. (1995)
[2] Data from Vitousek et al. (1995)
[3] Data from Herbert and Fownes (1999)

short period of litter collection was chosen to avoid leaching of polyphenols. The collected litter was combined across traps within each plot, air-dried in a custom made dry box, and then sorted into seven fractions: four different categories of *Metrosideros* leaves, leaves of other species, twigs, and other material (reproductive parts and parts too small to be identified). The four *Metrosideros* leaf fractions included 1) leaves that were entirely or partially green, 2) leaves that showed obvious signs of herbivory (mainly galls) regardless of leaf color, 3) dark brown and/or bleached leaves that had remained in the canopy after sensescence and death, and 4) yellow-brown, newly senesced leaves. In addition to the litter collected in traps, we also sampled the litter layer and surface soil at each site and within each plot. Partially decomposed *Metrosideros* leaf litter was collected from the deepest litter layer, but only entire leaves of an intact shape were sampled. Surface soil was collected after removal of the litter layer. These materials were air-dried in the same way as litter from the traps.

Fine root litter was collected from the same sites and plots as described above in December 1999. A total of three soil cores were randomly taken within each plot at all sites using a 5-cm diameter corer. A sampling depth of 15 cm was chosen because most of the fine roots are found within this part of the soil profile (Ostertag 2001). The soil cores were pooled within each plot, kept in a cool box in the field and then stored at 4 °C for a maximum of 4 days until they could be processed. Processing involved washing soil through a 1-mm sieve and separating roots from other organic material in a tray of deionized water. The resulting roots were separated into 1) live fine roots, 2) dead fine roots, 3) coarse roots, and 4) fern roots, and then air-dried. Fine roots are defined as < 2-mm diameter and coarse roots as > 2-mm diameter. Live and dead fine roots were distinguished based on color and texture (Vogt and Persson (1991), R. Ostertag, pers. comm.). Live roots were flexible and friable and were of light color, whereas dead roots were rigid and the stele often separated from the cortex. Coarse roots and fern roots were not separated into live and dead material, because they could not easily be distinguished (mainly fern roots) or because most of them appeared to be alive (coarse roots). Except for fern roots having a characteristic black color and morphology (uniform diameter and no lateral roots), we were unable to separate roots by species. However, after removal of fern roots, it can be assumed that most of the remaining roots were *Metrosideros polymorpha*, because of its high dominance at all sites.

In December 1999, we collected green, full-sun leaves of *Metrosideros* from small branches at the top of the canopy using either a shotgun or a slingshot. Between three and five leaves from the oldest leaf cohort were taken from each branch, and three or more trees were sampled in each site and plot. These leaves were air-dried in the same way as litter material.

The annual rate of phenolic input was estimated on a ground area basis using our measurements of polyphenol concentrations and measurements of annual above-ground litter production by Harrington et al. (2001) and estimates of annual below-ground litter production by Ostertag (2001). Harrington et al. (2001) collected above-ground litter fall from each plot of all four fertilization treatments at the Thurston and Kokee sites from June 1997 until June 1998. Root turnover rates were determined at the same sites, but only for the fertilization treatments C, N, and P (Ostertag 2001). Our estimates of total annual polyphenol inputs were restricted to the Thurston and Kokee sites. Below-ground polyphenol input for the NP treatments were estimated by assuming a similar ratio of above- to below-ground litter production among the different treatments within each site. Assuming a minimal turnover and resorption of phenolic compounds during leaf senescence, we defined the difference between green leaf phenolic production and leaf litter phenolic input as phenolic input by canopy throughfall.

Common garden plants

In 1996 *Metrosideros* seedlings were collected at all four sites along the natural fertility gradient (Treseder and Vitousek 2001). Ten plants from each location were planted into 8-L plastic pots containing a mixture of vermiculite, soil, and lava gravel. Potted seedlings were kept in the open at the University of Hawaii Volcano Experimental Farm in the Olaa Forest Reserve, and were watered regularly to prevent soil desiccation during dry spells (see Treseder and Vitousek (2001)).

We collected fresh-fallen leaf litter from mesh screens underneath each individual plant every three days until there was enough material (about 5 consecutive collections) in August 1999 and in May 2000. With a few exceptions (some leaves with galls) this litter was of the yellow-brown, newly senesced type. Additionally, five to eight full-sun green leaves from the oldest age class were collected from each individual plant in December 1999. Due to the relatively small size of the pots, we did not collect fine roots.

Analyses of polyphenols

Air-dried material from all collections was ground and subsequently used for all phenolic analyses. Phenolic compounds were extracted from a 20- to 40-mg sample with 10 mL of 50% ethanol during two hours, shaking every 15 minutes. This procedure was found to be most suitable after initial comparisons among different solvents (methanol, ethanol and acetone), and between different concentrations (50:50 solvent:water, 70:30 solvent:water) and different time spans of extraction (2 h and 24 h). The extract was then filtered through a Whatman glass microfibre filter using a vacuum filtration unit. The filtrate was analyzed for phenolic compounds using two analytical approaches. We measured total phenolics (TPh) using the Folin-Denis method as a general phenolic assay (Swain and Hillis 1959) that does not discriminate between tannin and non-tannin phenolics. With the acid butanol method (Porter et al. 1986) which is a specific functional group assay, we measured proanthocyanidins (syn. condensed tannins). The Folin-Denis assay is based on a redox reaction, and it thus measures not only phenolics but also other easily oxidized, non-phenolic material such as ascorbic acid (Hagerman et al. 1997). Commercially available tannic acid (CAS 1401-55-4) was used to standardize the Folin-Denis assay.

The acid butanol method is suitable for selective determination of proanthocyanidins (Hagerman and Butler 1989; Hagerman et al. 1997). The assay depends on the specific molecular structure of the proanthocyanidin, so we prepared a standard by purifying tannin from green, air-dried *Metrosideros* leaves (Hagerman and Butler 1980). Ground leaf material was extracted four times with 50% acetone (4 × 50 mL per 10 g leaf material) for 15 minutes each time. The collected extract was evaporated under reduced pressure (rotary evaporated) to remove acetone. The aqueous solution was thoroughly extracted with diethyl ether to remove most of non-polar compounds (mainly chlorophyll) using a separatory funnel. This step was repeated six times before the aqueous fraction again was evaporated under reduced pressure to remove small amounts of ether and to reduce the total volume. The remaining extract was diluted with absolute ethanol and then applied to Sephadex LH20 equilibrated in ethanol to chromatographically separate tannin from nontannin phenolics. After washing the Sephadex with absolute ethanol until the eluate was free of phenolics (based on absorbance at 280 nm), the tannin adsorbed to the chromatographic support was eluted with 50% aqueous acetone. The acetone was removed from the eluate by evaporation under reduced pressure, and the samples were then lyophilized to yield a light-brown powder.

The anthocyanidins produced by reaction of the purified *Metrosideros* tannin with acid butanol had a broad absorbance peak with a maximum absorbance at 553 nm, suggesting that the *Metrosideros* tannin is a mixed polymer of catechin (yields cyanidin with maximum absorbance at 547 nm) and gallocatechin (yields delphinidin with maximum absorbance at 557 nm). *Metrosideros* tannin did not release any galloyl groups on hydrolysis, suggesting that it is pure proanthocyanidin (condensed tannin) with no hydrolyzable tannins.

Although the Folin-Denis assay yields an estimate of the total concentration of all phenolics, including proanthocyanidins (PA), the estimate of PA from the acid butanol assay is usually not simply a subset of the total phenolics estimate. This is because the chemical reactions differ considerably between the two methods and the comparability of their yields depends on the structure of the specific phenolic analyte (Hagerman and Butler 1989).

Data analyses

Polyphenol concentrations of the various materials analyzed were compared among unfertilized control plots at the four sites along the long substrate age gradient, or among the fertilization treatments within each of the three sites with long-term fertilization experiments, with one-way ANOVA. To evaluate possible differences in fertilization effects among sites, we performed two-way ANOVA with site and fertilization treatment as fixed factors. Results of post-hoc multiple comparisons (Fisher's LSD test) are reported at $\alpha = 0.05$. In all analyses, the plots at each site were treated as replicates ($n = 4$). Whenever necessary, data were arc sin square root transformed to homogenize variances. Differences in polyphenol concentration between dead and live plant tissues taken from the same plots were compared using a paired t-test. All data were analyzed using SYSTAT (SYSTAT 1992).

Results

Metrosideros leaf litter comprised more than 55% of the total above-ground litter collected in the litter traps, when averaged across sites and dates of collection. Up to 75% of that *Metrosideros* leaf litter was of the yellow-brown, newly senesced type. The other three *Metrosideros* leaf litter fractions comprised roughly equal amounts of the remaining *Metrosideros* leaf litter. The concentrations of polyphenols did not differ among dates of collection, so we report data from the most extensive (December 1999) collections, unless otherwise noted.

Natural soil fertility gradient

Most of the measured litter materials differed significantly in their polyphenol concentrations along the soil chronosequence (Table 2). The concentration of TPh in newly senesced (yellow-brown) *Metrosideros* leaf litter was 65% higher at the oldest, phosphorus limited site (Kokee) compared to the youngest, nitrogen limited site at Thurston; the relatively fertile sites Laupahoehoe and Kohala showed intermediate concentrations (Figure 1). Litter from Thurston also had the lowest proanthocyanidin concentration, but the differences were much smaller than those observed for TPh.

Fine root litter generally had a lower concentration of TPh than leaf litter. Moreover, the relative differences among sites were different from those seen in leaf litter (Figure 1), with the lowest concentrations in fine root litter from Laupahoehoe and the highest concentrations at Thurston. Laupahoehoe also had the lowest concentration of proanthocyanidin. Polyphenol concentrations in fine roots generally did not correlate with those in leaves, regardless of whether live or dead tissues were considered (Figure 2).

The differences in polyphenol concentration among sites were less pronounced for all litter fractions combined than for newly senesced *Metrosideros* leaf litter alone (Table 2). Twig litter had relatively low polyphenol concentrations and partially decomposed *Metrosideros* leaves were essentially free of polyphenols at either site (Table 2). Absolutely no polyphenols could be extracted from topsoils at either site (data not shown).

Effects of long-term fertilization

Long-term fertilization with N, P, or both at Thurston, Laupahoehoe and Kokee did not significantly alter polyphenol concentrations in *Metrosideros* litter (Table 2). A significant response to fertilization, however, was observed in the tree fern *Cibotium* (Figure 3). Results of two-way ANOVAs showed a significant negative effect of fertilization on the concentrations of TPh (F = 3.99; P = 0.021) and PA (F = 7.27; P = 0.0013) in *Cibotium* leaf litter. This fertilization effect was due primarily to P additions, and was more pronounced at the N-limited site at Thurston than at the relatively fertile site at Laupahoehoe. Proanthocyanidin concentrations in tree fern litter from control plots at Laupahoehoe and Kohala were lower than that from

Table 2. Concentrations of total phenolics and proanthocyanidins in above- and below-ground litter. Plant material was collected across the long substrate age gradient on the Hawaiian islands (T = Thurston, L = Laupahoehoe, H = Kohala, K = Kokee) and from each of four different long-term fertilization treatments (Control, + N, + P, + NP) at three sites (T, L, K). All values are shown as percent of total air-dry mass (mean ± SE, n = 4). Different letters within a row (within sites) or column (across sites within one treatment) indicate significant differences ($P < 0.05$).

	Site	Total Phenolics				Proanthocyanidins			
		Control	+ N	+ P	+ NP	Control	+ N	+ P	+ NP
Bulk of above-ground litter	T	7.3 ± 0.6^{a}	7.5 ± 0.3^{a}	7.6 ± 0.4^{a}	7.9 ± 0.4^{a}	5.9 ± 0.6^{a}	6.8 ± 0.9^{a}	5.6 ± 0.3^{a}	5.5 ± 0.2^{a}
	L	6.3 ± 0.1^{a}	7.5 ± 0.4^{a}	7.3 ± 1.9^{a}	6.5 ± 0.3^{a}	5.8 ± 0.7^{a}	8.8 ± 0.7^{a}	6.4 ± 1.2^{a}	5.8 ± 1.1^{a}
	H	7.7 ± 0.6^{a}	–	–	–	5.5 ± 0.4^{a}	–	–	–
	K	14.5 ± 0.5^{b}	13.6 ± 0.8^{b}	12.6 ± 1.9^{b}	13.6 ± 0.7^{b}	7.6 ± 0.8^{a}	8.3 ± 1.2^{a}	6.6 ± 0.3^{a}	7.6 ± 0.4^{a}
Metrosideros fresh leaf litter	T	11.2 ± 0.4^{a}	11.4 ± 0.7^{a}	10.6 ± 0.7^{a}	11.1 ± 0.5^{a}	9.4 ± 0.4^{a}	11.1 ± 0.3^{ab}	9.7 ± 0.3^{ab}	10.5 ± 0.4^{ab}
	L	14.7 ± 0.7^{b}	14.2 ± 0.6^{b}	14.6 ± 0.6^{b}	15.1 ± 0.3^{b}	12.0 ± 0.7^{b}	10.9 ± 0.8^{b}	9.9 ± 1.1^{b}	11.8 ± 0.4^{b}
	H	13.5 ± 0.3^{b}	–	–	–	12.3 ± 0.7^{b}	–	–	–
	K	19.0 ± 0.8^{c}	19.3 ± 1.7^{c}	17.6 ± 0.4^{c}	17.8 ± 0.9^{c}	12.4 ± 0.7^{bc}	11.7 ± 0.8^{bc}	13.5 ± 1.2^{c}	11.5 ± 0.6^{bc}
Metrosideros part. decomposed leaf litter	T	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{ab}	0.3 ± 0.0^{ab}	0.3 ± 0.0^{a}
	L	0.5 ± 0.2^{b}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.5 ± 0.2^{b}	0.5 ± 0.1^{b}	0.4 ± 0.0^{b}	0.4 ± 0.1^{b}	0.3 ± 0.1^{a}
	H	0.6 ± 0.0^{b}	–	–	–	0.6 ± 0.1^{b}	–	–	–
	K	0.5 ± 0.2^{b}	0.5 ± 0.0^{b}	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}	0.6 ± 0.1^{bc}	0.6 ± 0.0^{c}	0.4 ± 0.1^{bc}	0.6 ± 0.0^{bc}
Metrosideros twig litter	T	1.0 ± 0.5^{a}	0.6 ± 0.2^{a}	0.6 ± 0.3^{a}	0.5 ± 0.1^{a}	2.6 ± 0.8^{a}	2.0 ± 0.4^{a}	2.2 ± 0.8^{ab}	2.1 ± 0.6^{ab}
	L	0.8 ± 0.2^{a}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.2^{a}	0.8 ± 0.6^{b}	0.8 ± 0.1^{b}	1.0 ± 0.1^{b}	1.1 ± 0.2^{b}
	H	0.2 ± 0.1^{a}	–	–	–	3.0 ± 0.4^{a}	–	–	–
	K	0.7 ± 0.2^{a}	0.6 ± 0.1^{a}	0.5 ± 0.1^{a}	0.5 ± 0.2^{a}	1.2 ± 0.6^{b}	0.9 ± 0.1^{b}	1.0 ± 0.1^{b}	1.1 ± 0.1^{b}
Metrosideros fine root litter	T	9.5 ± 0.9^{a}	10.5 ± 0.8^{a}	9.6 ± 0.7^{a}	11.3 ± 0.1^{a}	9.8 ± 0.3^{a}	9.0 ± 0.9^{a}	9.1 ± 0.6^{ab}	9.3 ± 0.6^{a}
	L	7.0 ± 0.8^{b}	8.0 ± 0.6^{b}	9.1 ± 1.1^{b}	7.8 ± 0.9^{b}	6.7 ± 1.0^{b}	6.5 ± 0.8^{ab}	7.4 ± 1.4^{b}	5.0 ± 0.9^{b}
	H	8.4 ± 0.2^{ab}	–	–	–	13.1 ± 0.5^{c}	–	–	–
	K	9.6 ± 0.4^{a}	8.8 ± 0.4^{ab}	9.5 ± 0.9^{ab}	8.6 ± 1.0^{ab}	14.1 ± 1.2^{c}	15.1 ± 1.6^{c}	13.8 ± 2.4^{ac}	13.3 ± 2.0^{ac}

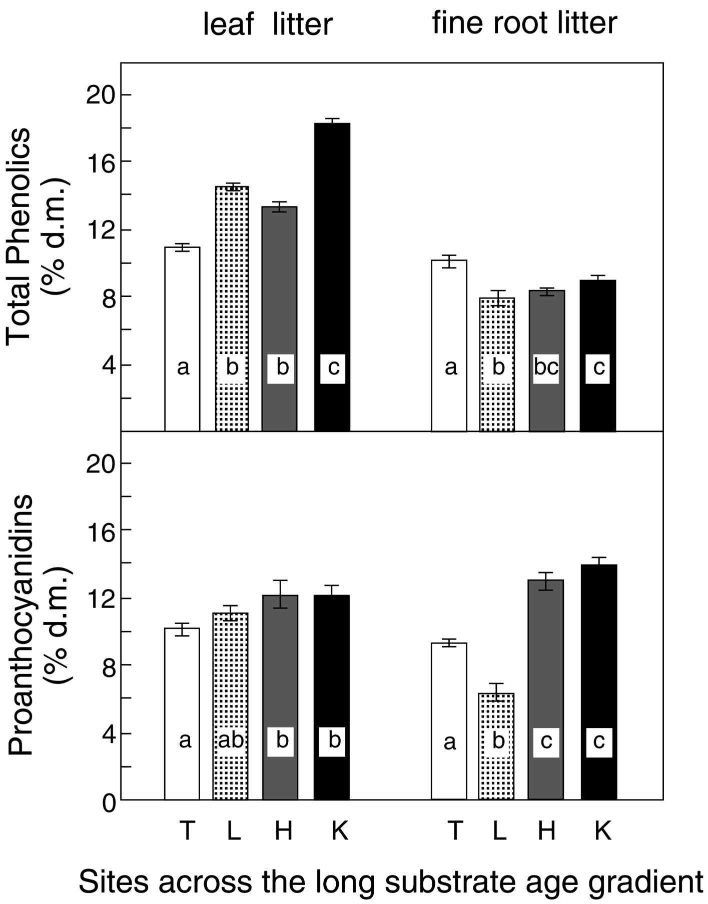

Figure 1. Concentrations of total phenolics (top panel) and proanthocyanidins (bottom panel) in *Metrosideros* leaf litter and fine root litter collected at four different sites across the long substrate age gradient (T: Thurston [0.3 kyr], L: Laupahoehoe [20 kyr], H: Kohala [150 kyr], K: Kokee [4100 kyr]). Mean values pooled across 4 different fertilization treatments (T, L, K; no significant fertilization effects) or 4 control plots (Kohala) are shown (± SE, in % of air-dry mass). Different letters indicate significant differences among sites within each measurement of phenolic compounds and within each tissue type at $P < 0.05$ using Fisher LSD post hoc contrasts.

control plots at Thurston, but similar to the concentrations measured in fertilized plots at Thurston (Figure 3). Because *Cibotium* was sparse at Kokee, a different fern species, *Elaphoglossum*, was sampled. Neither roots nor leaf litter of *Elaphoglossum* were affected by fertilization (data not shown).

The response of *Cibotium* roots to fertilization was generally weaker than that of leaf litter. *Cibotium* roots at Thurston had similar concentrations of TPh and PA among the different fertilization treatments, and those grown at Laupahoehoe showed a trend for lower concentrations with fertilization.

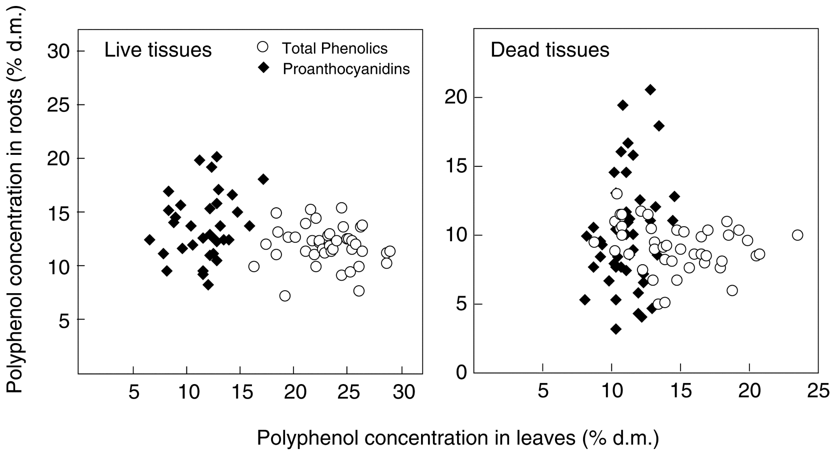

Figure 2. Polyphenol concentration (total phenolics: open circles, proanthocyanidins: black diamonds) in fine roots as a function of polyphenol concentration in leaves for either live tissues (left) or dead tissues (right) of *Metrosideros*. Each symbol represents the values of one plot from each of four fertilization treatments (except Kohala) from each of four sites across the long substrate age gradient. All values are in % of air-dry mass.

Comparisons with common garden plants

Comparisons of polyphenol concentrations in leaves of *Metrosideros* between natural populations and plants from the same populations but grown under identical conditions in a common garden are shown in Figure 4. Total phenolics in green leaves and in newly senesced leaf litter had significantly higher concentrations in common garden plants than in leaves from the natural populations. Despite these differences in absolute concentrations, the relative differences among sites were almost identical for plants grown either in the common garden or at the natural location, in particular for green leaves. The non significant location × site interactions (Table 3) also indicate that the concentrations of TPh in green leaves and leaf litter vary similarly across sites of origin, regardless where the plants grew.

The concentration of PA in green leaves and in leaf litter was similar in common garden plants and in plants grown at the natural locations (Figure 4, Table 3). However, the location × site interaction term explained relatively more variation in leaf litter than in green leaves.

Annual input rates of polyphenols from above-and below-ground litter

The total above- and below-ground input of phenolic compounds ranged from 62.4 to 170.8 g per square meter of ground per year for TPh and from 47.1 to 112.3 g/m^2/yr for PA (Figure 5). These numbers include an estimated contribution of canopy throughfall that makes up an average of 28% and 10% of the total input of TPh and PA. On average the input rates were significantly higher at Kokee than at Thurston for both TPh (F = 16.42; P = 0.0005) and PA (F = 10.98; P = 0.0029). In

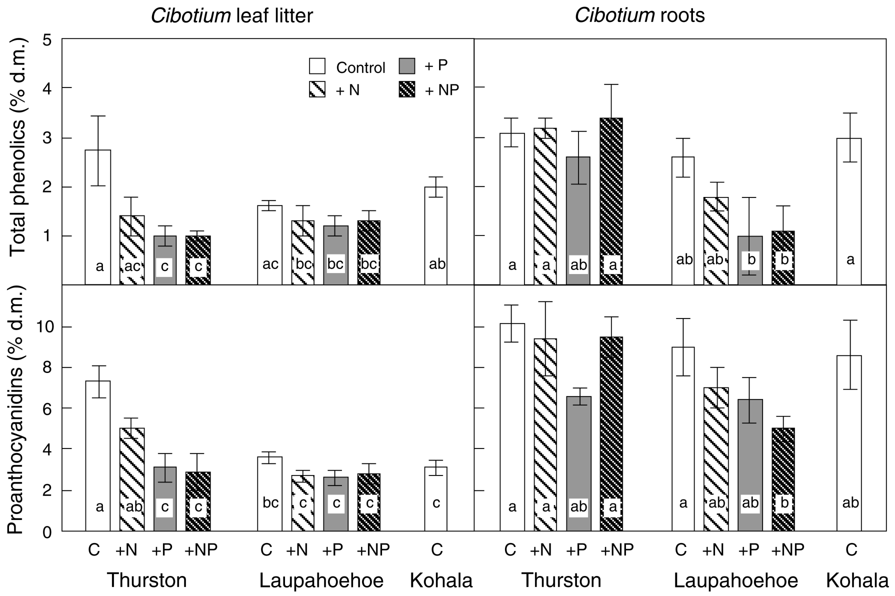

Figure 3. Concentrations of total phenolics (top) and proanthocyanidins (bottom) in leaf litter and fine roots (not distinguished between alive and dead) of *Cibotium* spp. grown at three different sites across the long substrate age gradient (*Cibotium* was too rare at Kokee) under four different long-term fertilization treatments (no fertilization at Kohala). Mean values of 4 different plots are shown (± SE, in % of air-dry mass). Different letters indicate significant differences among sites and fertilization treatments within each measurement of phenolic compounds and within each tissue type at $P < 0.05$ using Fisher LSD post hoc contrasts.

addition, the two sites differed considerably in the relative contribution of below-ground inputs (Figure 5). The input of TPh from root turnover accounted for 28% of the total amount at Thurston compared to only 11% at Kokee ($F = 191.15$; $P < 0.0001$). Likewise, PA from root litter contributed 40% at Thurston and 23% at Kokee to the total above- and below-ground proanthocyanidin input ($F = 46.73$; $P < 0.0001$). Despite having lower overall polyphenol input rates, Thurston showed significantly higher absolute rates of below-ground polyphenol inputs (both TPh and PA) than Kokee.

Discussion

Variation in litter polyphenol concentrations across a soil fertility gradient

Polyphenol concentrations in leaf litter and fine root litter varied independently across the soil age gradient, to the extent that they were uncorrelated, regardless whether live or dead tissues were considered. These results suggest that phenolic allocation to leaves and roots is affected differently by changing soil fertility. It is

Table 3. Analysis of variance of polyphenol concentration in green leaves and leaf litter of *Metrosideros* to test for effects of location (common garden vs. natural location), site of origin (T, L, H, K) and their interactions.

Polyphenols	Source of variance	df	Sum of Squares	Mean Square	*F*-value	*P*-value
Green leaves						
Total phenolics	Location	1	33.13	33.13	9.47	0.0038
	Site of origin	3	78.66	26.22	7.49	0.0004
	Loc × Site	3	0.89	0.30	0.08	0.97
	Error	40	139.95	3.50		
Proanthocyanidin	Location	1	0.38	0.38	0.08	0.78
	Site of origin	3	14.61	4.87	1.02	0.40
	Loc × Site	3	8.99	2.99	0.63	0.60
	Error	34	162.52	4.78		
Leaf litter						
Total phenolics	Location	1	167.16	167.16	76.67	0.0000
	Site of origin	3	151.44	50.48	23.15	0.0000
	Loc × Site	3	13.44	4.48	2.05	0.13
	Error	34	73.97	2.18		
Proanthocyanidin	Location	1	0.50	0.50	0.26	0.62
	Site of origin	3	11.85	3.95	2.01	0.13
	Loc × Site	3	15.22	5.07	2.58	0.07
	Error	34	66.47	1.96		

difficult to fit these findings to existing conceptual models designed to predict and understand the variability in plant polyphenol concentrations, such as the carbon nutrient balance (CNB) hypothesis (Bryant et al. 1983), the growth differentiation model (Herms and Mattson 1992), or the recent protein competition model (PCM, Jones and Hartley (1999)). While different in some important details, all of these models essentially operate at the physiological level of resource use and allocation by the plant, and generally predict higher phenolic allocation when nutrients are growth limiting. Increasing concentrations of total phenolics (TPh) and – to a lesser extent – of proanthocyanidins (PA) in *Metrosideros* leaf litter with increasing P limitation are in line with these predictions. However, our results are opposite the expected pattern based on N availability, in that the lowest polyphenol concentrations were found at the most N-limited site. It is tempting to speculate that N limitation is severe enough to constrain C gain and so synthesis of phenolics in the N-limited Thurston site, while P limitation constrains growth more than it does C gain. However, below-ground we observed the highest concentration of TPh from the N-limited site at Thurston and the lowest concentrations of both TPh and PA from the relatively fertile site at Laupahoehoe – a pattern consistent with model predictions. The contrasting patterns observed for leaves and fine roots suggest distinct underlying processes determining polyphenol concentrations in different organs of the same plant species, and question the general application of model predictions at the whole plant level.

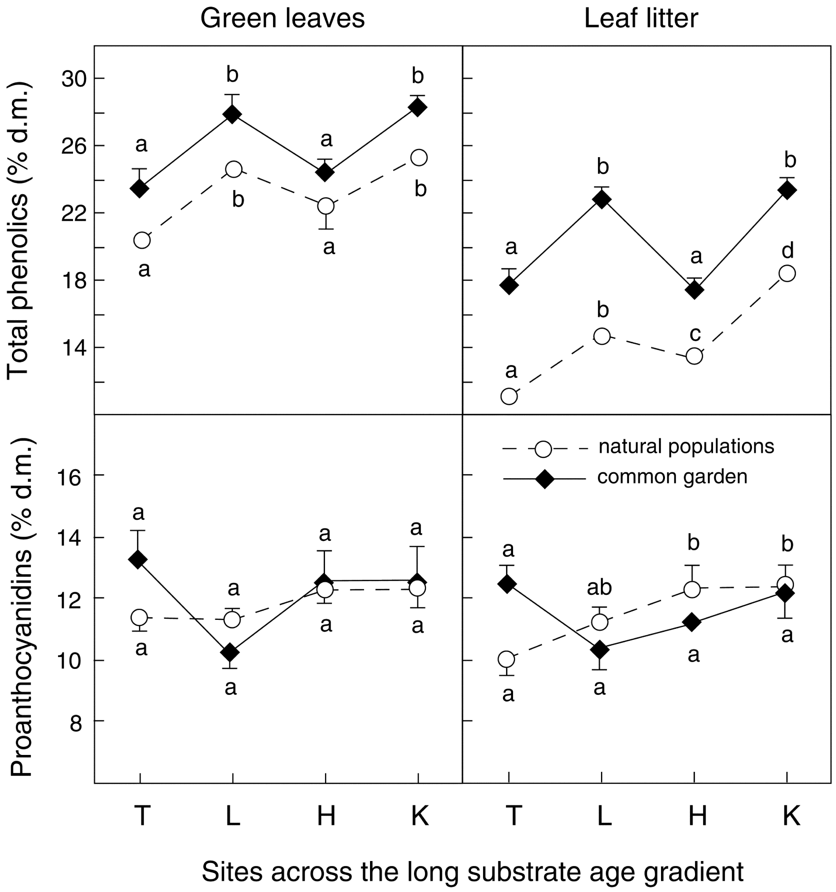

Figure 4. Concentrations of total phenolics (top) and proanthocyanidins (bottom) in green leaves (oldest leaf cohort) and leaf litter of *Metrosideros* grown either at the four different sites (open circles) or in a common garden (black diamonds). Symbols (± SE) represent mean values of n = 4 fertilization treatments (natural populations) or n = 8 individual trees (common garden). Different letters indicate significant differences among sites (T: Thurston [0.3 kyr], L: Laupahoehoe [20 kyr], H: Kohala [150 kyr], K: Kokee [4100 kyr]) within natural populations and within common garden plants at $P < 0.05$ using Fisher LSD post hoc contrasts.

Phenotypic versus genotypic variation

How can the observed differences in polyphenol concentrations in *Metrosideros* among sites be explained? An important step towards answering this question is to distinguish between phenotypic and genotypic controls. Our results strongly suggest that polyphenol concentrations inherently vary among *Metrosideros* populations, rather than being directly affected by nutrient availability at the level of the phenotype. Polyphenol concentrations in both live and dead leaves and fine roots did not change in response to long-term fertilization at either site, despite consid-

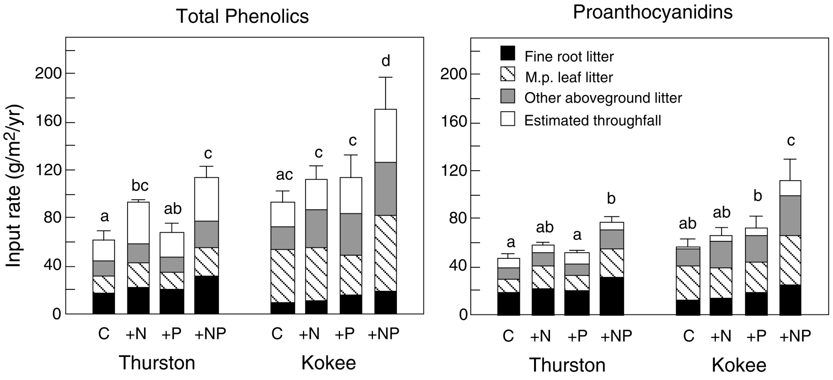

Figure 5. Estimated above- and below-ground annual input rates of total phenolics (left) and proanthocyanidins (right) in *Metrosideros* dominated Hawaiian montane forests at Thurston (N-limited) and Kokee (P-limited) under long-term fertilization (mean ± SE, n = 4). Throughfall is calculated as the difference in total production of phenolics based on green leaf phenolic concentration and that based on leaf litter concentration. Different letters indicate significant differences among sites and fertilization treatments at $P < 0.05$ using Fisher LSD post hoc contrasts.

erable increases in plant growth and NPP in response to N and P fertilization in the Thurston and Kokee sites, respectively (Vitousek et al. 1993; Herbert and Fownes 1995; Vitousek and Farrington 1997; Harrington et al. 2001; Ostertag 2001). This result contrasts with the predictions of the phenotypic PCM variant (Jones and Hartley 1999) and to previous studies reporting altered phenolic concentrations in green leaves after fertilization (e.g. Gershenzon (1984) and Bryant et al. (1987), Haukioja et al. (1998)). Proanthocyanidins in particular can respond strongly (Bryant et al. 1987; Keinänen et al. 1999), while nontannin phenolics might be less responsive (Keinänen et al. 1999). In contrast to *Metrosideros*, the dominant understory tree fern *Cibotium*, showed significantly reduced polyphenol concentrations under fertilization. Interestingly, P application reduced TPh and PA more than N fertilization, even when growth was limited more by N than by P.

Phenotypic responses that have been observed elsewhere also include phenolic production following herbivore attack (Karban and Baldwin 1997). We do not think that herbivory explains our results, at least not above-ground. The most abundant leaf herbivore on *Metrosideros* is a gall-forming psyllid, and there is no significant difference in gall abundance among our study sites (D. Gruner, personal communication). Moreover, newly senesced leaf litter with galls covering more than 50% of the leaf surface did not differ in polyphenol concentrations than similar leaves without any signs of herbivore damage (unpublished data). Overall, polyphenols in *Metrosideros* responded little to changes in abiotic and biotic factors. The lack of change in response to long-term fertilization, in particular, suggests that the control of polyphenol synthesis occurs at a level beyond short-term physiological processes that are shaped by resource availability.

The comparisons between natural populations of *Metrosideros* and common garden plants also suggest that the observed differences in litter polyphenol concentrations have a strong genetic component. Even though the absolute concentrations differed between field and garden for TPh, the relative differences among sites of origin were essentially the same in both. Similarly, a highly specific phenolic composition was demonstrated among genotypes of silver birch (*Betula pendula*) that also showed different phenolic responses to fertilization and defoliation (Keinänen et al. 1999). They suggested that genetic variation in the expression of phenolic composition and concentration may reduce the incidence of herbivore attack within a population of silver birch. We interpret our results as indicating genotypic adaptation to site-specific ecosystem properties. Since climate, altitude, and species composition are essentially the same among study sites, substrate age-related differences in soil fertility remains as the most likely selection force. However, it cannot be ruled out that some other unknown selection pressures were involved in the past, or that polyphenol concentrations co-vary with other plant traits that has been selected for.

Fate of polyphenols and functional implications

What is the fate and function of polyphenols within the soil, and how could they drive natural selection for polyphenol-rich plant genotypes on infertile soils? It has been found repeatedly that TPh and tannins are leached rapidly from litter, because essentially gone within the first two months after litterfall (Kuiters and Sarink 1986; Schofield et al. 1998). In line with these two studies, partially decomposed *Metrosideros* leaf litter measured here contained less than 1% of TPh and PA. At the same time, essentially no polyphenols could be extracted from the topsoil – in accordance with observations by Schofield et al. (1998). Because only a subset of some low molecular weight phenolics can be metabolized readily by soil microorganisms, most polyphenols are likely complexed with proteins and other organic compounds, and held in a form where they cannot be detected with the usual analytical procedures.

Polyphenol-protein complexes (PPC) make organically bound nitrogen less accessible for microbial mineralization. Although this may reduce N availability to some plants, it has been reported that some mycorrhizae have the capability to break down such polyphenol-protein complexes (PPC) transferring nitrogen from these complexes to their host plant (Bending and Read 1995). In addition, Northup et al. (1995b) observed that increasing amounts of dissolved organic nitrogen and decreasing amounts of mineral nitrogen were produced from decomposing pine needle litter with increasing polyphenol concentration. They suggested that polyphenol-rich litter might be an adaptation to N-limited soils that works by short-circuiting the microbial mineralization step and increasing plant uptake of organic N (see also Chapin (1995)). Based on this hypothesis the highest polyphenol concentrations would be expected at Thurston, the most N-limited site. While leaf litter at Thurston actually had the lowest concentrations, fine root litter had the highest TPh concentration of all the sites, and had a higher PA concentration than was found at the relatively fertile site at Laupahoehoe. Moreover, the absolute input rate of root-derived polyphenols per unit ground area was significantly higher at Thurston than at Kokee, even though the total amount of phenolic input (above- and below-ground) was considerably lower at Thurston. These results suggest that the distinct functional importance of leaf- and root-derived polyphenols might be further accentuated by their completely different spatial distribution within the soil profile. Below-ground phenolic input provides a comparatively even distribution of polyphenols across the soil profile with a high probability to bind proteins from the microbe-rich rhizosphere, a mechanism which might support the hypothesis of Northup et al. (1995b, 1998). However, the mechanisms linking plant polyphenol production, soil processes and plant fitness first need to be elaborated more thoroughly, before firm conclusions about soil fertility driven natural selection for polyphenol-rich plant genotypes can be drawn (Hättenschwiler and Vitousek 2000).

For the interpretation of ecosystem-level processes, the actual rate of polyphenol production per unit ground area is more informative than just the concentrations in litter. We estimated a total phenolic input of 83.0 g and 122.4 g of TPh, and 58.5 g and 77.0 g of PA per square meter and per year (mean across fertilization treatments) at Thurston and Kokee, respectively. These numbers probably are overestimates because 10% (PA) to 28% (TPh) of this amount is contributed by "throughfall" which is not actually measured, but calculated as the difference between polyphenol content in the oldest age class of green leaves and leaf litter. In the other published study of phenolic input we located, it was estimated that the total phenolic input was 20.4 g/m^2/yr in a sugar maple dominated temperate forest ecosystem (McClaugherty 1983). This estimate includes only the above-ground leaf litter contribution, in our study it compares to an average (across both the Thurston and Kokee sites) of 55.6 g/m^2/yr of total phenolics. The nearly threefold higher phenolic input in our study is consistent with the notion that tropical montane forests have a particularly high phenolic production compared to other forest ecosystems (Bruijnzeel and Veneklaas 1998).

Acknowledgements

We are grateful to: the Divisions of Forestry and Wildlife and State Parks of the State of Hawaii, Hawaii Volcanoes National Park, the Joseph Souza Center at Kokee State Park, and Parker Ranch at Waimea for access to the field sites and logistical support; H. Farrington, J. Moen and D. Penn for invaluable field and laboratory assistance; D. Turner for the help with chemical analyses and laboratory support; R. Ostertag for advice on root sampling; D. Gruner and K. Treseder for discussion and comments on drafts of this manuscript. This research was supported by a USDA-NRI research grant to Stanford University and a postdoctoral research fellowship to S. Hättenschwiler from The Swiss National Science Foundation, The Novartis Foundation, and The Holderbank Foundation.

References

Bate-Smith E.C. 1962. The phenolic constituents of plants and their taxonomic significance: I Dicotyledons. J. Linn. Soc. 58: 95–173.

Bending G.D. and Read D.J. 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behaviour and translocation of nutrients from exploited litter. New Phytologist 130: 401–409.

Bruijnzeel L.A. and Veneklaas E.J. 1998. Climatic conditions and tropical montane forest productivity: the fog has not lifted yet. Ecology 79: 3–9.

Bryant J.P., Chapin F.S. III and Klein D.R. 1983. Carbon-nutrient balance of boreal plant in relation to vertebrate herbivory. Oikos 40: 357–368.

Bryant J.P., Clausen T.P., Reichardt P.B., McCarthy M.C. and Werner R.A. 1987. Effects of nitrogen fertilisation upon the secondary chemistry and nutritional vlaue of quaking aspen (*Populus tremuloides* Michx) leaves for the large aspen tortrix (*Choristoneura conflicta* (Walker)). Oecologia 73: 513–517.

Chapin F.S. III 1995. New cog in the nitrogen cycle. Nature 377: 199–200.

Coley P.D., Bryant J.P. and Chapin F.S. III 1985. Resource availability and plant antiherbivore defense. Science 230: 895–899.

Crews T.E., Kitayama K., Fownes J.H., Riley R.H., Herbert D.A., Mueller-Dombois D. et al. 1995. Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence. Ecology 76: 1407–1424.

Findlay S., Carreiro M., Krischik V. and Jones C.G. 1996. Effects of damage to living plants on leaf litter quality. Ecol. Appl. 6: 269–275.

Gallet C. and Lebreton P. 1995. Evolution of phenolic patterns in plants and associated litters and humus of a mountain forest ecosystem. Soil Biol. Biochem. 27: 157–166.

Gershenzon J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. Rec. Adv. Phytochem. 18: 273–320.

Giambelluca T.W., Nullet M.A. and Schroeder T.A. 1986. Rainfall Atlas of Hawaii. Department of Land and Natural Resources State of Hawai'i.

Grime J.P., Cornelissen J.H.C., Thompson K. and Hodgson J.G. 1996. Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. Oikos 77: 489–494.

Hagerman A.E. 1988. Extraction of tannin from fresh and preserved leaves. J. Chem. Ecol. 14: 453–462.

Hagerman A.E. and Klucher K.M. 1986. Tannin-protein interactions. In: Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure-Activity Relationships. Alan R Liss Inc, pp. 67–76.

Hagerman A.E. and Butler L.G. 1980. Condensed tannins: Purification and characterization of tannin-associated proteins. J. Agric. Food Chem. 28: 947–952.

Hagerman A.E. and Butler L.G. 1989. Choosing appropriate methods and standards for assaying tannin. J. Chem. Ecol. 15: 1795–1810.

Hagerman A.E., Zhao Y. and Johnson S. 1997. Methods for determination of condensed and hydrolyzable tannins. In: Shahidi F. (ed.), Antinutrients and Phytochemicals in Food ACS Symposium, Series 662. American Chemical Society.

Harborne J.B. 1997. Plant secondary metabolism. In: Crawley M.J. (ed.), Plant Ecology. 2nd edn. Blackwell Science, Oxford, pp. 132–155.

Harrington R.A., Fownes J.H. and Vitousek P.M. 2001. Production and resource use efficiencies in N- and P-limited tropical forests: A comparison of responses to long-term fertilization. Ecosystems 4: 646–657.

Hartley S.E. and Jones C.G. 1997. Plant chemistry and herbivory: or why the world is green. In: Crawley M.J. (ed.), Plant Ecology. 2nd edn. Blackwell Science, Oxford, pp. 284–324.

Haslam E. 1989. Plant polyphenols Vegetable tannins revisited. Cambridge University Press, Cambridge.

Hättenschwiler S. and Schafellner C. 1999. Opposing effects of elevated CO_2 and N deposition on *Lymantria monacha* larvae feeding on spruce trees. Oecologia 118: 210–217.

Hättenschwiler S. and Vitousek P.M. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol. Evol. 15: 238–243.

Haukioja E., Ossipov V., Koricheva J., Honkanen T., Larsson S. and Lempa K. 1998. Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization? Chemoecol. 8: 133–139.

Herbert D.A. and Fownes J.H. 1995. Phosphorus limitation of forest leaf area and net primary productivity on a highly weathered soil. Biogeochem. 29: 223–235.

Herbert D.A. and Fownes J.H. 1999. Forest productivity and efficiency of resource use across a chronosequence of tropical montane soils. Ecosystems 2: 242–254.

Herms D.A. and Mattson W.J. 1992. The dilemma of plants: to grow or defend. Quat. Rev. Biol. 67: 283–335.

Horner J.D., Gosz J.R. and Cates R.G. 1988. The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. Am. Nat. 132: 869–883.

Jones C.G. and Hartley S.E. 1999. A protein competition model of phenolic allocation. Oikos 86: 27–44.

Karban R. and Baldwin I.T. 1997. Induced responses to herbivory. University of Chicago Press, Chicago.

Keinänen M., Julkunen-Tiitto R., Mutikainen P., Walls M., Ovaska J. and Vapaavouri E. 1999. Trade-offs in phenolic metabolism of silver birch: effects of fertilization, defoliation, and genotype. Ecology 80: 1970–1986.

Kitayama K. and Mueller-Dombois D. 1995. Vegetation changes during long-term soil development in the Hawaiian montane forest zone. Vegetatio 120: 1–20.

Kuiters A.T. 1990. Role of phenolic substances from decomposing forest litter in plant-soil interactions. Acta. Botanica. Neerl. 39: 329–348.

Kuiters A.T. and Sarink H.M. 1986. Leaching of phenolic compounds from leaf and needle litter of several deciduous and coniferous trees. Soil Biol. Biochem. 18: 475–480.

Lamb C.J., Lawton M.A., Dron M. and Dixon R.A. 1989. Signals and transduction mechanisms of activation of plant defenses against microbial attack. Cell 56: 215–224.

McClaugherty C.A. 1983. Soluble polyphenols and carbohydrates in throughfall and leaf litter decomposition. Acta Oecol. – Oec. Gen. 4: 375–385.

Northup R.R., Dahlgren R.A. and Yu Z. 1995a. Intraspecific variation of conifer phenolic concentration on a marine terrace soil acidity gradient; a new interpretation. Plant and Soil 171: 255–262.

Northup R.R., Yu Z., Dahlgren R.A. and Vogt K.A. 1995b. Polyphenol control of nitrogen release from pine litter. Nature 377: 227–229.

Northup R.R., Dahlgren R.A. and McColl J.G. 1998. Polyphenols as regulators of plant-litter-soil interactions: a positive feedback. Biogeochem. 42: 189–220.

Ostertag R. 2001. The effects of nitrogen and phosphorus availability on fine root dynamics in Hawaiian montane forests. Ecology 82: 485–499.

Porter L., Hrstich T. and Chan C.R. 1986. Acid butanol proanthocyanidin assay. Phytochem. 25: 223–230.

Ruohomäki K., Chapin F.S. III, Haukioja E., Neuvonen S. and Suomela J. 1996. Delayed inducible resistance in Mountain birch in response to fertilization and shade. Ecology 77: 2302–2311.

Schofield J.A., Hagerman A.E. and Harold A. 1998. Loss of tannins and other phenolics from willow leaf litter. J. Chem. Ecol. 24: 1409–1421.

Swain T. 1979. Tannins and lignins. In: Rosenthal G.A. and Janzen D.H. (eds), Herbivores. Academic Press, New York, pp. 657–682.

Swain T. and Hillis W.E. 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic compounds. J. Science Food Agric. 10: 63–68.

SYSTAT 1992. SYSTAT: Statistics, version 521 edition Systat. SYSTAT, Evanston, IL, USA.

Treseder K. and Vitousek P.M. 2001. Potential ecosystem-level effects of genetic variation among populations of *Metrosideros polymorpha* from a soil fertility gradient in Hawaii. Oecologia 126: 266–275.

Vitousek P.M. and Farrington H. 1997. Nutrient limitation and soil development: Experimental test of a biogeochemical theory. Biogeochem. 37: 63–75.

Vitousek P.M., Turner D.R. and Kitayama K. 1995. Foliar nutrients during long-term soil development in Hawaiian montane rain forest. Ecology 76: 712–720.

Vitousek P.M., Walker L.R., Whiteaker L.D. and Matson P.A. 1993. Nutrient limitation to plant growth during primary succession in Hawaii Volcanoes National Park. Biogeochem 23: 197–215.

Vogt K.A. and Persson H. 1991. Measuring growth and development of roots. In: Lassoie J.P. and Hinckley T.M. (eds), Techniques and approaches in forest tree ecophysiology. CRC Press, Boca Raton, FL, USA, pp. 477–501.