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Extracellular Enzyme Activities and Carbon Chemistry as Drivers of Tropical Plant Litter Decomposition[1]

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ABSTRACT

Litter quality parameters such as nitrogen and lignin content correlate with decomposition rates at coarse scales, but fine-scale mechanisms driving litter decomposition have proven more difficult to generalize. One potentially important driver of decomposition is the activity of extracellular enzymes that catalyze the degradation of complex compounds present in litter. To address the importance of this mechanism, we collected 15 Hawaiian plant litter types and decomposed them in fertilized and control plots for up to two years. We measured litter nutrient content and carbon chemistry prior to decomposition, as well as extracellular enzyme activities, mass loss, and litter nutrient content over time. We found that water-soluble carbon content, cellobiohydrolase activities, and polyphenol oxidase activities were significantly correlated with mass loss. Enzyme activities and decomposition rate constants both varied significantly by litter type, and fertilization increased mass loss rates in five litter types. Some litter types that decayed faster under fertilization also showed time-dependent increases in carbon-degrading enzyme activities, but others decayed faster independent of enzyme changes. These results suggest that extracellular enzyme activities partially determine litter decomposition rates, but high soluble carbon content may circumvent the requirement for enzyme-catalyzed decomposition.

Key words: *decomposition; enzymes; Hawaii; litter quality; nitrogen; phosphorus; tropical wet forest.*

THE DECOMPOSITION AND MINERALIZATION OF PLANT MATERIAL strongly influences ecosystem nutrient cycling rates and carbon (C) balance (Olson 1963, Rustad 1994, Schlesinger & Lichter 2001). Rapid release of nutrients from decaying plant litter may increase soil fertility, whereas recalcitrant litter immobilizes available nutrients and reduces rates of nutrient cycling and plant growth (McGrath *et al.* 2000, Kwabiah *et al.* 2001). In many soils, the accumulation of residual organic matter can benefit plants by decreasing bulk density and increasing cation exchange and water-holding capacity (Sollins *et al.* 1996). The balance between rates of decomposition and net primary production also controls soil C stocks and the potential for ecosystems to sequester anthropogenic CO_2 (Potter & Klooster 1997, Richter *et al.* 1999, Freeman *et al.* 2001, Schlesinger & Lichter 2001).

Because litterfall represents a large flux of C and nutrients, many studies have examined the mechanisms that regulate litter decomposition (Tenney & Waksman 1929, Aber *et al.* 1990, Sinsabaugh *et al.* 1993, Hobbie 1996, Berg 2000). The majority of litter mass is comprised of insoluble compounds that require enzymatic activity to decompose, and recent studies have explored the relationship between microbial extracellular enzymes and litter decomposition (Sinsabaugh *et al.* 1993, 2002; Sinsabaugh & Moorhead 1994). Based on the results of an experiment with decaying birch sticks, Sinsabaugh and Moorhead (1994), developed an extracellular enzyme allocation model to relate enzyme activities and litter decomposition rates. The model predicted that decomposition rates would be higher when nitrogen (N) and phosphorus (P) were more abundant because microbes could allocate more resources to C-degrading enzymes that limit litter decomposition.

While the model worked well for a common substrate (birch sticks) decomposing in different sites, it was less useful for predicting decomposition rates from enzyme activities in different litter types (Sinsabaugh *et al.* 2002). Across litter types, litter-specific chemical and physical properties can obscure the relationship between enzyme activity and decay rates by altering the effectiveness and stability of microbial enzymes. For example, low litter surface area and high toughness may limit enzyme diffusion and microbial access (Cornelissen 1996, Cornelissen *et al.* 1999). An abundance of soluble compounds in litter can increase mass loss rates through leaching, independent of enzyme activities (Schofield *et al.* 1998), while polyphenols present in litter can bind to proteins and reduce enzyme activity (Appel 1993). Thus, the relationship between potential enzyme activities and decomposition rates may not be direct because many factors

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can alter enzyme activities after the enzymes are produced (Burns 1982, Sinsabaugh 1994).

To evaluate the relationship between enzymes, litter properties, and decomposition rates, we selected 15 Hawaiian litter types that differed strongly in quality and chemical composition and compared their enzyme dynamics and mass loss rates. By measuring enzyme activities in combination with mass loss, nutrient dynamics, and initial litter quality, we sought to identify the litter parameters that affect decomposition rates by altering enzyme effectiveness. We also applied N + P fertilizer to decomposing litter to test whether microbial enzyme allocation patterns would shift under higher nutrient availability and cause faster decomposition (Sinsabaugh & Moorhead 1994).

We expected that litter types with the most favorable combinations of litter physical and chemical properties would decay the fastest, and that decomposition rates would be related to extracellular enzyme activities. We also anticipated that fertilization and high concentrations of endogenous nutrients in litter would cause microbes to produce more C-degrading enzymes, thereby speeding up litter decomposition (Sinsabaugh & Moorhead 1994).

MATERIALS AND METHODS

Litter collection.—We collected 15 litter types representing 11 plant species in July 2000 from sites on the islands of Hawaii, Oahu, and Kauai (Table 1). Litter was collected by harvesting senesced leaves from the plant or retrieving recently fallen leaves from the ground. Stem material was collected similarly for species that lost leaves and stems as a unit (*i.e.*, fern rachii). Each litter sample was air-dried to constant mass and thoroughly homogenized before subsampling. Subsamples were withdrawn to determine air-dry/oven-dry (70°C) mass conversion ratios and initial litter properties. The remaining litter was divided into 2 g subsamples and placed in 1 mm mesh fiberglass window screen bags, which allowed access by most soil invertebrates (Hobbie & Vitousek 2000).

Site description and design.—In September 2000, we deployed 960 litterbags in a 1 ha site at 150 m elevation on the island of Hawaii (19°40′N, 155°4′W). Rainfall is abundant (3500–4500 mm/yr) and aseasonal (Giambelluca *et al.* 1986), and the substrate is 1400-year-old basalt with shallow, poorly developed soils and a patchy litter layer and root mat. Mean annual temperature is 23°C, and the site vegetation is a *Psidium cattleianum*- and *Metrosideros polymorpha*-dominated, closed-canopy forest with a mixture of native and exotic trees and understory plants.

Four fertilizer and four control plots (5 × 5 m) were established at the site on flat terrain. Each plot received five bags of each litter type to monitor mass loss and nutrient dynamics and three bags to monitor enzyme activities. Fertilizer was applied at a rate of 100 kg/ha N and 100 kg/ha P at the initiation of the experiment and 50 kg/ha N and 50 kg/ha P at 4, 8, and 16 months after initiation (Vitousek & Farrington 1997). Litter collections occurred at 14, 47, 86, 152, and 204 days (labile litter); 14, 47, 86, 204, and 402 days (intermediate litter); or 14, 86, 204, 402, and 735 days (recalcitrant litter). Enzyme bags were collected with the first, second, and third or fourth mass loss bags and frozen until analysis (usually within one month and never longer than three months). Although the site experiences strong rain events, we did not account for enzyme loss due to leaching because the vast majority of extracellular enzymes are tightly bound to organic substrates (Burns 1982). After removing roots and soil particles, litter from mass loss bags was oven-dried (70°C), weighed, and ground in a Wiley mill for nutrient analyses.

Litter chemistry.—We analyzed total N and P content in litter using Kjeldahl digestion followed by colorimetric assay on an Alpkem autoanalyzer (OI Analytical, Wilsonville, Oregon). Samples were run with NIST 1515 apple leaves as standards, and recoveries ($\bar{x} \pm$ SD) were 95 ± 8 percent for N and 96 ± 9 percent for P. Four replicate oven-dried (70°C) and ground initial litter subsamples were analyzed for lignin content using acetyl bromide digestion (Iiyama & Wallis 1990) and NBS pine as a standard (lignin content = 21.8%). We analyzed another set of four replicate, air-dried initial litter subsamples for total phenolics using the Folin assay (AOAC 1950) and condensed tannins using the butanol–HCl assay (Mole & Waterman 1987). To account for varying reactivities of phenolics in different plant species, we constructed separate standard curves for each litter species using tannins purified from a composite sample of the initial litter (Appel *et al.* 2001). While the purified tannins generated linear standard curves for the Folin assay, some litter types did not contain enough condensed tannin to generate a standard curve for the butanol–HCl method. For these litter types, we report the condensed tannin content as 0 percent. A final oven-dried initial litter subsample was sent to

TABLE 1. *Species names (F = fern, D = dicot, M = monocot), specific leaf area (SLA), nutrient contents, and total mass loss for the 15 litter types.* N = *5–7 for SLA;* N = *3–4 for litter nutrients and percent mass lost at final collection.*

Litter Type	SLA (cm^2/g) $\bar{x}$ (SE)	Litter N (mg/g) $\bar{x}$ (SE)	Litter P (mg/g) $\bar{x}$ (SE)	Time of Final Collection (d)	Percent Mass Lost (Control) $\bar{x}$ (SE)	Percent Mass Lost (Fertilized) $\bar{x}$ (SE)
Stem material						
Cibotium glaucum (F)		1.9 (0.1)	0.35 (0.08)	402	41.6 (10.4)	50.6 (3.2)
Dicranopteris linearis (F)		1.2 (0.1)	0.06 (0.02)	735	31.8 (1.9)	28.3 (2.6)
Sphaeropteris cooperi (F)		3.4 (0.1)	0.51 (0.01)	402	45.5 (4.4)	48.0 (4.0)
Hedychium gardnerianum (M)		2.1 (0.2)	0.28 (0.07)	204	58.7 (11.3)	78.3 (7.4)
Ferns						
Cibotium glaucum	114 (8)	7.5 (0.2)	0.35 (0.01)	735	64.8 (7.5)	71.3 (10.5)
Dicranopteris linearis	112 (7)	5.3 (0.4)	0.25 (0.02)	735	32.7 (9.0)	43.5 (14.6)
Diplazium sandwichianum	227 (19)	14.2 (0.8)	0.80 (0.04)	402	77.9 (7.6)	75.2 (5.8)
Elaphoglossum alatum	95 (5)	4.9 (0.2)	0.19 (0.01)	735	60.7 (5.8)	70.3 (4.9)
Sphaeropteris cooperi	183 (5)	12.9 (0.3)	1.18 (0.03)	402	90.0 (3.7)	93.4 (1.3)
Angiosperms						
Vaccinium calycinum (D)	144 (2)	5.6 (0.5)	0.28 (0.04)	402	86.2 (3.7)	82.5 (5.2)
Hedychium gardnerianum (M)	167 (12)	3.8 (0.0)	0.26 (0.02)	402	78.3 (9.8)	93.8 (3.4)
Setaria palmifolia (M)	237 (11)	6.8 (0.5)	0.37 (0.02)	402	88.8 (4.2)	93.5 (4.5)
Clidemia hirta (D)	204 (19)	7.5 (0.2)	0.27 (0.01)	204	90.2 (1.3)	93.8 (1.2)
Miconia calvescens (D)	202 (13)	10.2 (0.9)	0.54 (0.04)	204	78.1 (5.5)	94.9 (1.3)
Tibouchina herbacea (D)	267 (12)	7.8 (0.1)	0.45 (0.01)	204	91.9 (2.9)	97.3 (1.4)

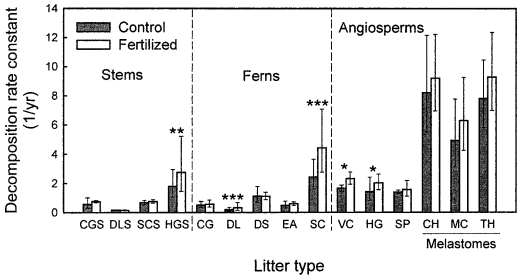

FIGURE 1. Mean (±95% CI) decomposition rate constants by treatment and litter type. $N = 4$. Significant fertilizer effect denoted by (*) $P < 0.1$, (**) $P < 0.05$, (***) $P < 0.01$. *x*-axis labels: CGS = *Cibotium* stems, DLS = *Dicranopteris* stems, SCS = *Sphaeropteris* stems, HGS = *Hedychium* stems, CG = *Cibotium glaucum*, DL = *Dicranopteris linearis*, DS = *Diplazium sandwichianum*, EA = *Elaphoglossum alatum*, SC = *Sphaeropteris cooperi*, VC = *Vaccinium calycinum*, HG = *Hedychium gardnerianum*, SP = *Setaria palmifolia*, CH = *Clidemia hirta*, MC = *Miconia calvescens*, and TH = *Tibouchina herbacea*.

the Center for Water and the Environment (Natural Resources Research Institute, University of Minnesota, Duluth, Minnesota) for C-fraction analysis using a standardized forest products procedure (Ryan *et al.* 1989). Fractions are reported on an ash-free basis and include nonpolar extractives (NPE; fats, oils, and waxes), water solubles (WS; amino acids, simple sugars, and soluble phenolics), acid solubles (AS; cellulose, hemicellulose, starch, polypeptides,and nucleic acids), and acid insolubles (AIS; mostly lignin). WS phenolics are reported as tannic acid equivalents, and WS and AS sugars are reported as glucose equivalents (DuBois *et al.* 1956).

Enzyme activities.—We measured extracellular enzyme activities in decomposing litter with assay techniques modified from Sinsabaugh *et al.* (1993). One-half of each moist litter sample was dried at 70°C and reweighed to determine water content, and the other half was coarsely chopped with scissors and assayed for the activity of three hydrolases and one oxidase: acid phosphatase (AP: releases free PO_4^{3-} by hydrolyzing phosphate ester bonds), N-acetyl-β-glucosaminidase (NAG: catalyzes chitin degradation), cellobiohydrolase (CBH: releases disaccharides from cellulose), and polyphenol oxidase (PPO: catalyzes oxidation of polyphenols).

Samples were combined with 60 ml 50 mM sodium acetate buffer, pH 5, and homogenized in a blender for one (leaf litter) or two minutes (stem litter). In a 2 ml centrifuge tube, 0.75 ml homogenate was combined with 0.75 ml substrate (AP: 5 mM pNP-phosphate, pNP = p-nitrophenol; NAG: 2 mM pNP-acetyl-β-D-glucosaminide; CBH: 2 mM pNP-cellobioside; PPO: 5 mM L-dihydroxyphenylalanine [DOPA]; all in 50 mM acetate buffer) and shaken vigorously for one to five hours at room temperature. Following centrifugation, we assayed the supernatant colorimetrically either for pNP (AP, NAG, and CBH reactions), or for the L-DOPA oxidation product (PPO reaction). We included appropriate controls to account for the background absorbance of the substrate and homogenate. For hydrolase reactions, we generated standard curves of absorbance versus concentration using known quantities of pNP (Sigma 104-1). The PPO reaction was standardized by using a commercial PPO preparation (Sigma T7755) to completely oxidize a known amount of L-DOPA and then measuring the absorbance of the reaction product.

Enzyme activities were initially calculated in units of μmol substrate consumed per gram dry weight per hour and then summarized as cumulative and relative activities to facilitate comparison across litter types, treatments, and different enzymes. As a measure of the total enzyme activity acting on a litter type during the experiment, we calculated cumulative values by performing a simple linear integration of daily enzyme activities through the last enzyme collection (Sinsabaugh *et al.* 2002). Because cumulative activities are biased by the duration of decomposition, we also calculated relative enzyme activities by dividing the cumulative activities by the total time of decomposition and then by the average activity value for each different enzyme. This calculation yields a time-averaged activity value that reflects the dominance of the enzyme in a litter type.

Statistical Analyses.—To facilitate comparison among litter types, we estimated annual litter decomposition constants (k-values) for each litter type in each plot by fitting mass loss over time to the negative exponential model (Olson 1963). We then subjected the calculated k-values to analysis of variance (ANOVA) with fertilization treatment and litter type as main effects. Because enzyme activities and nutrient concentrations were also related to litter type, we conducted simple and forward stepwise multiple regressions to determine if these continuous variables explained the variation in k-values. We performed a similar regression analysis to test the effects of initial litter parameters on decomposition rates using average k-values from control plots only. To determine which factors were controlling enzyme activities, we performed repeated-measures analysis of

TABLE 2. *Carbon chemistry of the 15 litter types. All values are percentages.* N = *3–4 for values with SE indicated;* N = *1 for values without standard errors. NPE = nonpolar extractives; WS = water solubles; AS = acid solubles; AIS = acid insoluble; TAE = tannic acid equivalents; GE = glucose equivalents.*

Litter Type	Lignin Content $\bar{x}$ (SE)	Phenolic Content $\bar{x}$ (SE)	Condensed Tannins $\bar{x}$ (SE)	NPE	WS	AS	AIS	TAE	GE (WS)	GE (AS)
Stem material										
Cibotium glaucum	29.4 (1.6)	3.5 (0.8)	1.43 (0.29)	2.3	13.6	60.7	23.4	2.6	3.6	49.5
Dicranopteris linearis	32.8 (1.5)	2.7 (0.4)	0.47 (0.14)	1.5	5.7	60.6	32.3	1.4	1.2	51.7
Sphaeropteris cooperi	36.5 (2.5)	1.0 (0.2)	0.13 (0.02)	1.9	6.1	61.9	30.1	0.5	1.7	50.1
Hedychium gardnerianum	11.9 (0.3)	7.9 (1.3)	0.00	4.4	16.7	74.9	4.0	0.2	1.8	66.0
Ferns										
Cibotium glaucum	18.2 (1.4)	6.7 (0.5)	2.41 (0.10)	8.7	13.4	39.1	38.7	3.8	4.1	22.2
Dicranopteris linearis	16.5 (1.6)	2.9 (0.8)	1.19 (0.40)	7.6	10.7	52.5	29.2	1.7	2.6	32.9
Diplazium sandwichianum	8.7 (1.0)	5.1 (0.5)	0.00	6.1	15.6	49.7	28.6	3.4	1.3	22.9
Elaphoglossum alatum	11.7 (1.3)	5.1 (1.8)	4.18 (1.43)	4.9	13.7	55.7	25.8	5.4	3.1	38.4
Sphaeropteris cooperi	16.2 (1.0)	3.5 (0.1)	1.60 (0.22)	6.4	13.7	45.7	34.1	3.7	4.3	21.7
Angiosperms										
Vaccinium calycinum	19.2 (1.8)	11.2 (0.9)	7.55 (0.83)	9.5	20.1	46.0	24.4	7.6	3.8	27.6
Hedychium gardnerianum	11.8 (0.1)	10.8 (1.5)	0.00	13.7	16.7	63.1	6.5	0.6	2.0	43.5
Setaria palmifolia	16.5 (0.9)	6.7 (0.6)	0.00	5.5	14.9	66.8	12.8	1.4	2.1	46.8
Clidemia hirta	17.3 (1.2)	20.1 (1.9)	0.00	5.8	41.8	42.4	10.0	22.2	6.5	12.7
Miconia calvescens	17.6 (1.0)	8.8 (2.1)	0.00	8.7	19.7	51.7	19.9	5.3	2.2	14.8
Tibouchina herbacea	19.2 (0.5)	13.0 (0.9)	2.25 (0.13)	9.7	33.7	49.2	7.4	12.8	4.8	15.0

TABLE 3. *Repeated measures ANCOVA results for litter extracellular enzyme activities. CBH = cellobiohydrolase; PPO = polyphenoloxidase; NAG = N-acetylglucosaminidase; AP = acid phosphatase. Significant* P *-values are highlighted in bold text.*

		CBH ($N = 347$)		PPO ($N = 347$)		NAG ($N = 347$)		AP ($N = 347$)	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment (T)	1	0.05	0.818	0.51	0.476	2.14	0.147	25.92	**<0.001**
Litter Type (L)	14	20.14	**<0.001**	30.47	**<0.001**	22.13	**<0.001**	56.40	**<0.001**
T × L	14	1.34	0.200	0.84	0.630	1.41	0.163	1.15	0.329
Time (TI)	3	4.59	**0.004**	31.37	**<0.001**	6.39	**<0.001**	49.98	**<0.001**
T × TI	3	4.26	**0.006**	0.82	0.484	2.61	0.0531	9.62	**<0.001**
L × TI	26	9.68	**<0.001**	4.26	**<0.001**	4.71	**<0.001**	10.27	**<0.001**
T × L × TI	26	0.94	0.551	1.32	0.154	1.35	0.133	0.54	0.966
Litter percent N	1	2.78	0.097	0.02	0.880	4.96	**0.027**	0.19	0.661
Litter percent P	1	0.00	0.975	0.23	0.632	1.60	0.208	0.00	0.994

covariance (ANCOVA; spatial power covariance structure) on all four measured enzyme activities using treatment, litter type, and time as main factors and litter N and P content as covariates. Prior to analyses, we removed obvious outliers from the data sets and log- or square root-transformed data when necessary to improve normality. For *post hoc* mean separations, we used Tukey's HSD test without correction for multiple comparisons so that we could detect marginally significant differences. We used SAS (version 8.2, SAS Institute) for all analyses; k-values were generated with PROC NLIN, regressions were performed using SAS PROC REG, and analyses of (co)variance were performed with PROC MIXED or PROC GLM.

RESULTS

Litter decomposition rates varied by litter type over two orders of magnitude, with exponential decay constants from less than 0.2 to greater than 9 per year ($F_{14,\ 105} = 188.2$, $P < 0.001$ for litter type effect; Fig. 1). Except for *Hedychium* stems and *Sphaeropteris* leaves, stem and fern material decayed slowly, while angiosperm leaves decayed rapidly (>5/yr for the Melastomataceae; Fig. 1). Fertilization with N and P significantly increased decomposition rates overall ($F_{1,\ 118} = 26.8$, $P < 0.001$) and there was no significant interaction with litter type, although a number of species were not significantly affected by the treatment (Fig. 1).

The initial litter characteristics of specific leaf area (SLA), N and P concentrations, percent lignin, percent phenolics, and percent condensed tannin all varied significantly by litter type ($P < 0.001$, one-way ANOVAs; Tables 1 and 2). Initial litter C fractions also varied widely by litter type (Table 2), although these data could not be statistically tested. Stem material was low in quality, with N and P contents below 3.4 and 0.51 mg/g, respectively (Table 1), and high concentrations of lignin and acid insoluble material (Table 2), although *Hedychium* stems contained only 4 percent insoluble compounds. Fern leaf litter was variable in quality with nutrient contents ranging from 4.9 to 14.2 mg/g N and 0.19 to 1.18 mg/g P, and acid insoluble materials greater than 25 percent (Tables 1 and 2). Angiosperm litter nutrient contents were similar to ferns but less variable (Table 1), and water-soluble C compounds (15–42%) rivaled or exceeded lignin and acid insoluble material (Table 2). For all litter types, final mass losses (Table 1) exceeded soluble content, showing that decomposition proceeded well past the initial leaching phase.

Extracellular enzyme activities differed strongly according to litter type and time, but the effect of fertilization was weaker and depended on time (Table 3). This pattern is shown clearly in Figures 2 and 3, where enzyme activities are integrated over time and differences among litter types are much greater than differences due to fertilization. Cumulative and relative enzyme activities were lowest in stems, but similar across fern and angiosperm leaf litter types. *Sphaeropteris* showed very high cumulative (51.4 mmol/g DW) and relative (73.6 units) CBH activity (Figs. 2a and 3a), while relative PPO activity was greatest in *Diplazium* (55.9 units in control plots; Fig. 3a). Cumulative NAG was greatest in *Elaphoglossum* (96.4 mmol/g DW; Fig. 2c), but relative activity was also high in most angiosperm litter types (Fig. 3a). *Tibouchina* showed the highest relative AP activity (77.5 units; Fig. 3a), but due to its rapid decay, cumulative activity was greater in other litter types, especially *Cibotium* and *Elaphoglossum* (Fig. 2d). The significant effects of time and litter × time on all four enzymes (Table

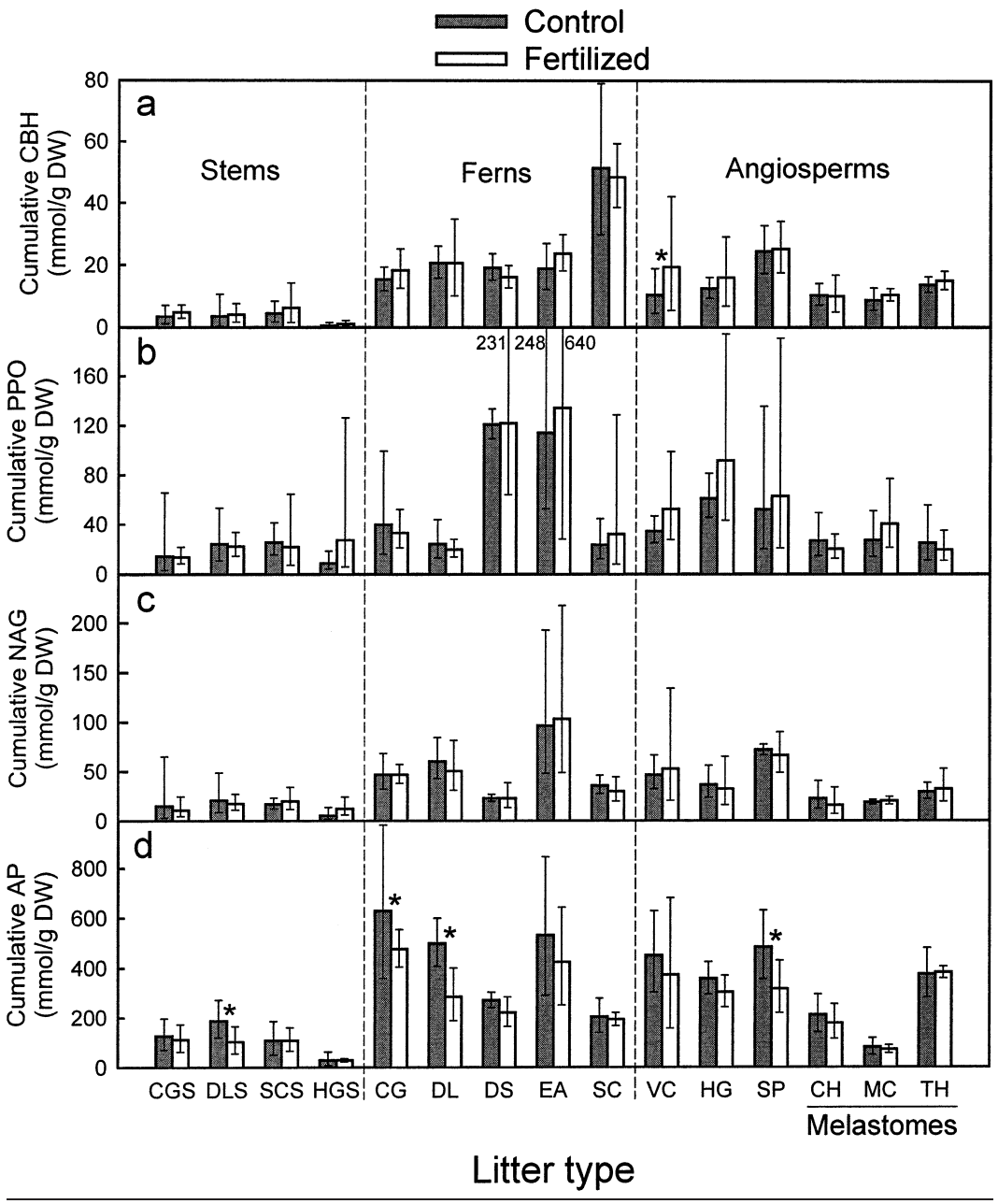

FIGURE 2. Mean (±95% CI) cumulative enzyme activities for (a) cellobiohydrolase, (b) polyphenol oxidase, (c) N-acetylglucosaminidase, and (d) acid phosphatase by treatment and litter type. $N = 4$. Enzyme activities in μmol/h/g DW were converted to daily rates and then integrated linearly over the time until the last enzyme collection (in days). Values in (b) indicate off-scale upper confidence limits. Significant fertilizer effect denoted by (*) $P < 0.05$. *x*-axis labels as in Fig. 1.

3) probably resulted from microbial succession and the utilization of different substrates as each litter type decomposed (Fioretto *et al.* 2000).

With the exception of AP, enzyme responses to fertilization with N and P were relatively minor. AP activity declined sharply in response to fertilization, especially in later stages of decomposition, which caused up to 45 percent reductions in cu-

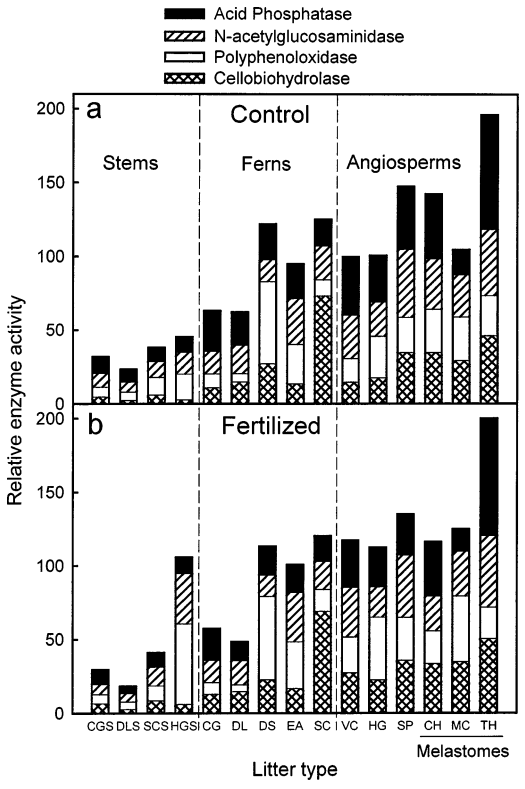

FIGURE 3. Relative enzyme activities for (a) control and (b) fertilized plots by litter type. Enzyme activities were scaled to the mean value for each enzyme, integrated over the time until the last enzyme collection (in days), and divided by the time of the last enzyme collection (in days) to obtain average daily activities in relative units that are comparable across different enzymes and litter types. *x*-axis labels as in Fig. 1.

mulative (Fig. 2d) and relative (Fig. 3b) AP activities. Fertilization increased CBH activity, but only at earlier times during decomposition (Table 3 and Fig. 4), leading to minimal changes in cumulative and relative enzyme activities (Figs. 2a and 3b). NAG and PPO activities were not significantly affected by fertilization. Carbon-degrading enzymes, however, may still mediate decomposition responses to fertilization in some litter types; CBH and PPO activities were elevated for specific periods of time during decomposition in three of the five litter types that decayed faster under fertilization (Fig. 4).

Simple linear regressions of decomposition rates versus litter enzymes activities and nutrient concentrations during decomposition did not reveal any strong relationships (Table 4). Most of the relative enzyme and litter nutrient variables explain small but statistically significant amounts of variation in decomposition rates when considered individually. When tested in a forward stepwise multiple regression, only positive relationships with relative CBH and PPO activities are significant, and they explain 52 percent of the variance in k-values.

Using a separate regression analysis with average k-values from control plots, we found that initial litter C chemistry was a better predictor of decay rates than enzyme activities or nutrient concentrations. Table 5 shows that five C chemistry variables explain significant amounts of variation in k-values according to simple regressions; however, a multiple regression shows that water-soluble C content alone is significantly and positively correlated with decay rates, explaining 72 percent of the variation. Because calculated k-values encompass a large fraction of mass loss for most litter types (Table 2), soluble C content explains decay rates even late in decomposition when lignin is expected to exert primary control. Other compounds that typically inhibit decomposition also had little effect here—polyphenol contents were high in some litter types, especially the Melastomataceae (9–20%), but these polyphenols were largely water-soluble and probably leached out early in decomposition (Table 2). Condensed tannins are more likely to slow down decomposition, but they were only detectable in eight litter types and concentrations were generally low (<3%) in the fast decaying litter types (Table 2).

DISCUSSION

Litter decomposition requires the solubilization of complex molecules through dissolution or microbe-mediated catalysis. Based on the model of Sinsabaugh and Moorhead (1994), we expected to find a positive linear relationship between CBH and

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FIGURE 4. Mean (lower 95% confidence limit) cellobiohydrolase (left panels) and polyphenol oxidase (right panels) activities by fertilization treatment and date for litter types that decayed at least marginally significantly faster ($P < 0.1$, Tukey's HSD test) under fertilization. Upper 95 percent confidence limits are omitted for clarity. $N = 4$. Collection dates varied for different litter types (see Methods), and are labeled as "Date 1–3" to facilitate comparison. Significant fertilizer effect denoted by (*) $P < 0.05$. ND = not determined.

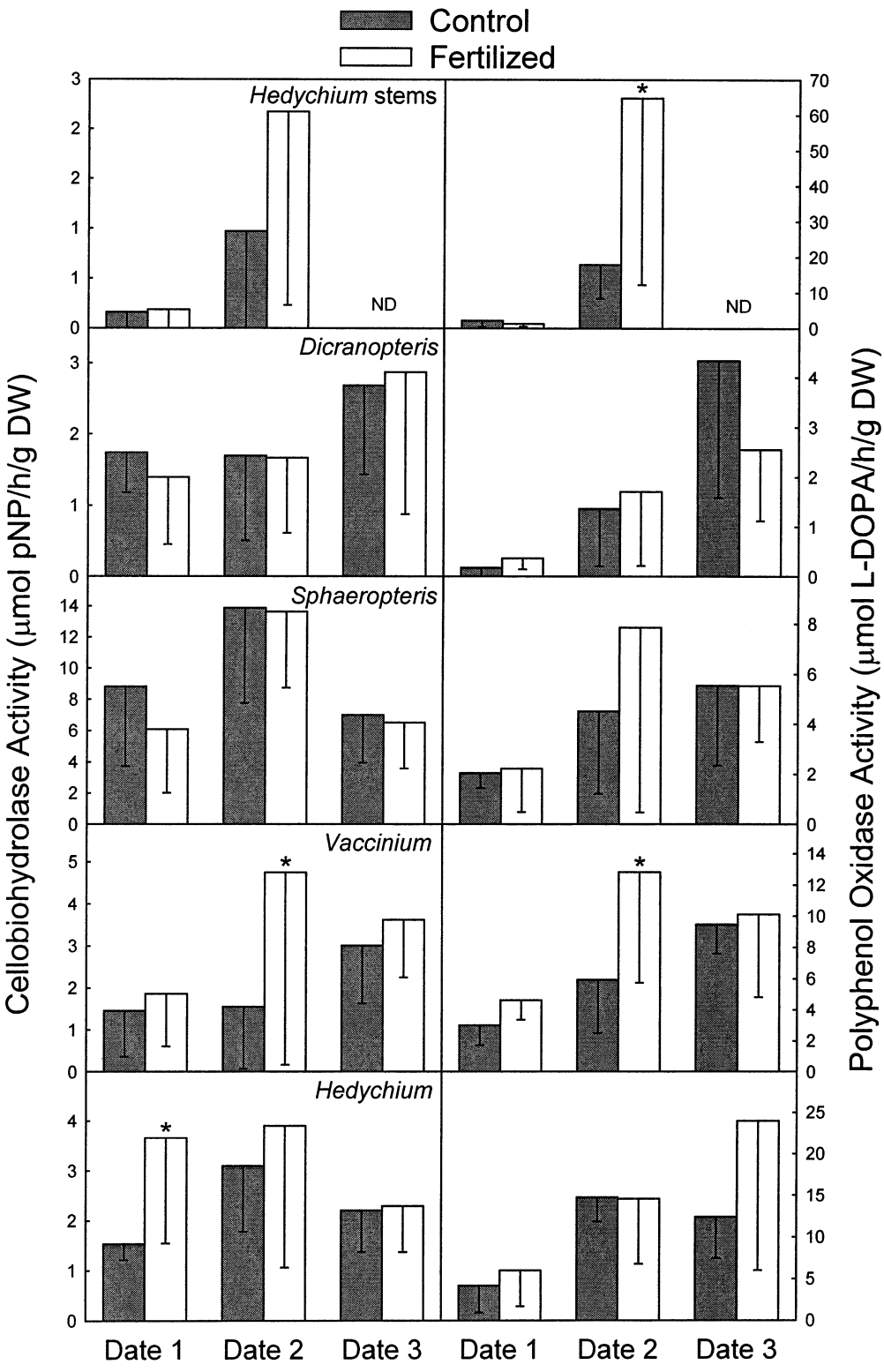
Control
Fertilized
Hedychium stems
Dicranopteris
Sphaeropteris
Vaccinium
Hedychium
ND
ND
*
Cellobiohydrolase Activity (µmol pNP/h/g DW)
Polyphenol Oxidase Activity (µmol L-DOPA/h/g DW)
Date 1
Date 2
Date 3
Date 1
Date 2
Date 3

TABLE 4. *Simple regression statistics for relative enzyme and nutrient concentration effects on decomposition k-values.* N = *120.*

Parameter	Effect on k-value	R^2	*P*-value
Relative CBH	+	0.43	<0.001
Relative PPO	+	0.30	<0.001
Relative NAG	+	0.33	<0.001
Relative AP	+	0.30	<0.001
Average litter percent N	+	0.11	<0.001
Average litter percent P	+	0.07	0.003

PPO activity and litter decomposition rates. Instead, decay rates were only weakly related to C-degrading enzyme activities and were more strongly related to water-soluble C content. In many of our litter types, mass loss probably occurred independent of enzyme activities, as soluble C compounds leached rapidly from the litter (Yavitt & Fahey 1986, Blum & Shafer 1988, Kuiters 1990, Ibrahima *et al.* 1995, Schofield *et al.* 1998). For example, *Clidemia* and *Tibouchina* litter contained 42 and 34 percent water-soluble compounds, respectively, half of which were phenolics (Table 2). Phenolics, however, can be resistant to microbial degradation, and litter mass loss rates may be greater than actual decomposition rates if these compounds are transferred from litter into soil C pools.

Surprisingly, water-soluble C content was a more important determinant of k-values than more common measures of litter quality, such as lignin and N content (Melillo *et al.* 1982, Berg 2000). Several types of fern litter decayed very slowly, despite having intermediate N contents, and most of the rapidly decomposing angiosperm litter types contained nearly 20 percent lignin and only moderate amounts of N. Acid-insoluble C contents, another measure of lignin, were lower but still substantial in the rapidly decaying litter types. Thus, the range of variation in lignin content was not large enough to account for the observed 50-fold difference in k-values, and litter N data were inconsistent with decay patterns. The lack of a strong relationship between lignin, N, and decomposition probably reflects the unique suite of litter types employed in our study, but also suggests that other studies should consider water-soluble C as a potential control on decay rates.

In contrast to our predictions based on previous studies (Sinsabaugh *et al.* 1993, Sinsabaugh & Moorhead 1994, Schimel & Weintraub 2003), we found that litter enzyme activities and decomposition rates were not well correlated; however, this result was not entirely unexpected because strong differences in litter chemical and physical characteristics can overwhelm the direct relationship between extracellular enzyme concentrations and decomposition rates. In recalcitrant stem litter and fern leaves, enzymes may be present but unable to catalyze decomposition because they cannot physically interact with their substrates (Burns 1982, Sinsabaugh *et al.* 1991). Lignin and other acid-insoluble compounds (Table 2) may shield enzyme substrates, thereby reducing enzymatic efficiency and shifting control of mass loss rates toward other mechanisms, such as leaching. Sinsabaugh *et al.* (2002) also found that enzymatic efficiencies varied widely among decomposing oak, maple, and dogwood litter according to differences in initial litter properties.

Based on modeling by Sinsabaugh and Moorhead (1994), we expected that fertilization would shift microbial resource allocation from nutrient acquisition to C acquisition, thereby increasing

TABLE 5. *Initial litter parameters explaining a significant amount of variation in decomposition k-values from control plots.* N = *15 for all variables except SLA where* N = *11.*

Parameter	Effect on k-value	R^2	*P*-value
SLA	+	0.56	0.008
Total phenolic content	+	0.58	<0.001
Water-soluble C	+	0.72	<0.001
Acid-insoluble C	−	0.38	0.015
Water-soluble sugars	+	0.27	0.045
Acid-soluble sugars	−	0.28	0.041

CBH and PPO activities relative to NAG and AP. Within a given litter type, this shift in enzyme allocation should cause an observable increase in decomposition rate. We did observe higher decomposition rates in response to fertilization, especially in species such as *Hedychium*, *Dicranopteris*, *Sphaeropteris*, and *Vaccinium* (Fig. 1). While AP activity declined as expected, CBH and PPO increases under fertilization appeared insufficient to cause observed increases in decay rates—in fact, *Dicranopteris* and *Sphaeropteris* decayed faster under fertilization with no significant change in CBH or PPO activities (Fig. 4). While fertilizer-induced increases in these enzymes might drive faster decay in some litter types (*e.g.*, *Hedychium* and *Vaccinium*), fertilization probably also affects decomposition through CBH- and PPO-independent mechanisms. Xylanases or other extracellular enzymes could be more important (Kandeler *et al.* 1999), or fertilization may increase decomposition through enzyme-independent processes.

Based on studies in temperate ecosystems (Berg 1986, Fog 1988), we expected that fertilizer-induced suppression of soil fungi would further slow decomposition in recalcitrant litter types such as *Cibotium*, *Dicranopteris*, *Elaphoglossum*, and stem material (Carreiro *et al.* 2000). Our results, however, do not support this prediction because we did not observe reduced PPO activity or decomposition rates under fertilization, and *Dicranopteris* actually decayed faster when fertilized (Fig. 1). Hobbie (2000) found a similar result for high-lignin *M. polymorpha* litter decomposing in a Hawaiian tropical forest. Thus, it is possible that fungi are not dominant members of the decomposer community in some Hawaiian ecosystems, or that responses of fungi to fertilization are ecosystem-dependent.

Overall, our results highlight the need for a better understanding of the mechanistic link between microbial extracellular enzyme production and litter decomposition. Across different litter substrates and even within a substrate under differing nutrient conditions, it may be difficult to detect the enzyme–decomposition relationship predicted by well-established conceptual models (Sinsabaugh & Moorhead 1994). In our study, differences in soluble C content overwhelmed the effect of extracellular enzymes on litter mass loss. Enzymatic action, however, is obviously necessary to degrade complex and insoluble litter compounds, and this decomposition mechanism remains poorly understood.

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