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Soil minerals and humic acids alter enzyme stability: implications for ecosystem processes

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Abstract In most ecosystems, the degradation of complex organic material depends on extracellular enzymes produced by microbes. These enzymes can exist in bound or free form within the soil, but the dynamics of these different enzyme pools remain uncertain. To address this uncertainty, I determined rates of enzyme turnover in a volcanic soil with and without added enzymes. I also tested whether or not soil minerals and humic acids would alter enzyme activity. In soils that were gamma-irradiated to stop enzyme production, 35–70% of the enzyme activity was stable throughout the 21-day incubation. The remaining enzyme fraction decayed at rates ranging from -0.032 to -0.628 day^{-1}. In both the irradiated soils and in soils with added enzymes, addition of the mineral allophane had a strong positive effect on most enzyme activities. Another added mineral, ferrihydrite, had a weak positive effect on some enzymes. Added humic acids strongly inhibited enzyme activity. These findings suggest that minerals, especially allophane, enhance potential enzyme activities in young volcanic soils. However, the actual activity and function of these enzymes may be low under field conditions if stabilization results in less efficient enzyme-substrate interactions. If this is the case, then much of the measured enzyme activity in bulk soil may be stabilized but unlikely to contribute greatly to ecosystem processes.

Keywords Allophane · Decomposition · Extracellular enzyme · Ferrihydrite · Microbe · Sorption

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Introduction

Extracellular enzymes enable soil microbes to degrade complex substrates into low molecular weight compounds that can be assimilated for growth (Schimel and Bennett 2004; Sinsabaugh 1994). Therefore, constraints on enzyme production and activity may regulate carbon (C) degradation and the release of nutrients from complex compounds (Allison 2006). Within the soil, a variety of biotic and abiotic factors regulate enzyme stability and interactions between enzymes and substrates.

Enzymes in soil differ in their origin, function, and turnover times (Burns 1982). This heterogeneity arises because soils contain a diversity of mineral and organic compounds that interact with soil enzymes (Sinsabaugh 1994). Many decades of research have revealed that mineral compounds generally have two opposing effects on enzyme

activity. As early as the 1940s, it was demonstrated that clay minerals could stabilize proteins against proteolysis and degradation (Ensminger and Gieseking 1942). Subsequent studies confirmed that enzymatic proteins were also protected from degradation as a result of mineral stabilization (Ladd 1978). However, enzyme stabilization on mineral surfaces represents a tradeoff: mineral-bound enzymes show altered kinetic properties that often reduce catalytic activity (Gianfreda and Bollag 1996; Kobayashi and Aomine 1967; Ladd 1978). These effects may include reduced enzyme V_{max} and increased K_m resulting from occlusion of the active site and sorption of substrate molecules (Quiquampoix et al. 2002; Sarkar et al. 1989; Tietjen and Wetzel 2003).

In many soils derived from volcanic parent material, the formation of non-crystalline minerals such as allophane and ferrihydrite occurs early in soil development (Shoji et al. 1993). These compounds are particularly abundant in young soils of the Hawaiian Islands, where non-crystalline minerals comprise 30–70% of total mineral content (Vitousek et al. 1997). Because allophane and other reactive minerals can complex and stabilize organic material (Schwertmann and Taylor 1989; Wada 1989), they may have particularly strong effects on the stability and activity of proteins, including enzymes.

Humic compounds are also important for enzyme function because they make up a large fraction of organic matter (Olk et al. 1995) and like minerals, may stabilize or inhibit enzyme activity (Nannipieri et al. 1996). Humic acids inhibit enzyme activity through complexation or covalent binding reactions that impede substrate access to the enzyme active site (Butler and Ladd 1971; Ladd and Butler 1969; Verma et al. 1975). As with minerals, these inhibitory effects can be at least partially offset by increased resistance of the enzyme to degradation (Nannipieri et al. 1996). Such interactions among enzymes and humic acids are important in both terrestrial and aquatic ecosystems (Wetzel 1991).

Recently, researchers have begun to recognize the fundamental role of enzymes in decomposition processes, and have incorporated enzymes into models (Allison 2005; Schimel and Weintraub 2003; Sinsabaugh and Moorhead 1994; Vetter et al. 1998). However, all these models consider only a single pool of enzyme with a uniform turnover time, and few studies have examined the sizes and functions of different enzyme pools that are known to exist within soils (Burns 1982). The dynamics of these enzyme pools should depend heavily on the mineral and organic composition of soils. Thus, the objectives of this study were to determine the turnover rates of enzymes within a well-characterized volcanic soil, and to examine the effects of common soil compounds on enzyme stability and activity. I hypothesized that ferrihydrite, allophane, and humic acids would stabilize enzymes against degradation in the soil, but would also suppress enzyme activity relative to control soils without added compounds.

Materials and methods

Soils

Soils were collected on the Island of Hawaii from a 300 year-old volcanic substrate at 1176 m elevation (Crews et al. 1995). The site has a mean annual temperature of 16°C and receives 2500 mm precipitation annually. Soils are classified as Lithic Hapludands and contain modest amounts of non-crystalline minerals, including allophane and immogolite (Vitousek et al. 1997). For use in laboratory incubations, I collected and combined 20 cores (6 cm diameter × 10 cm depth) from a 50 m transect, as described by Allison and Vitousek (2005). The soils were subsampled for water content and used on a dry weight basis in all subsequent analyses.

Soil mineral synthesis

I prepared ferrihydrite by titrating 20 mM ferric nitrate with 1 M NaOH to a pH of 7.0 and washing and freeze-drying the resulting precipitate. The precipitate was then ground to a fine powder with a mortar and pestle. Allophane was synthesized using a procedure modified from Wada et al. (1979). I hydrolyzed tetraethyl orthosilicate in deionized water under rapid mixing for 45 min to produce 2.0 mM silicic acid

(H_4SiO_4) and then added 224.5 ml of 0.1 M $AlCl_3$ to 11 l of silicic acid solution under rapid mixing. This solution was titrated with 716 ml of 0.1 M NaOH at a rate of ~7.5 ml min^{-1} followed by refluxing at 95–98°C for 5 days. The allophane precipitate was concentrated by flocculation with 500 ml of 4.0 M NaCl followed by centrifugation. Residual salts were removed by dialysis, and excess water was separated from the allophane gel by centrifugation. A subsample of the gel was dried at 70°C to determine water content.

Humic acid extraction

I extracted the humic acid fraction (hereafter, "humic acids") from a 1:1:1 mixture (dry weight) of surface soils (top 10 cm) from the 300 y, 20 ky, and 4.1 million y sites of the Hawaiian soil chronosequence described in Crews et al. (1995). Humic acids were extracted using strong base extraction, followed by acid precipitation and dialysis to remove salts according to Swift (1996). The purified humic acids were freeze-dried and powdered with a mortar and pestle before use in incubations.

Irradiation experiment

I examined the turnover of native enzymes by gamma-sterilizing soils and monitoring the decline in enzyme activity over time in soils with and without added soil minerals or humic acids. This approach halts enzyme production by the microbial community while allowing enzyme loss to occur via proteolysis, sorption, chemical degradation, or other abiotic pathways. However, an important consideration is that irradiation will also halt protease production. Eliminating protease production may cause enzyme degradation to decline, and therefore enzyme stability may be overestimated.

Using a glass rod, I mixed allophane, ferrihydrite, or humic acid into the soil at rates of 7.5, 30, and 15% respectively (dry weight equivalents). Soils were irradiated with 30 kGy gamma irradiation from a cesium-137 source and confirmed to be sterile by plating out on nutrient agar and verifying a lack of microbial growth. Enzyme activities were assayed 1, 3, 10, and 21 days after irradiation.

Enzyme addition experiment

To assess the turnover and stabilization of free enzymes, I added commercially available enzymes to soil amended with varying amounts of synthetic minerals or humic acid. Allophane, ferrihydrite, and humic acid were mixed into duplicate 0.5 g soil aliquots at rates of 0, 3.8, 7.5, 15, and 30% of soil mass (dry weight equivalents). I then added 59 units acid phosphatase (AP), 329 units β-glucosidase (BG), 249 units polyphenol oxidase (PPO), 44 units *N*-acetyl-glucosaminidase (NAG), and 1.18 units urease (UR). These amounts were chosen to ensure that a measurable amount of added enzyme activity would be present in all treatments late in the incubation. I focused on these five enzymes because they are active in C (BG, PPO), nitrogen (UR, NAG), and phosphorus (AP) cycling in a wide range of soils (Kandeler et al. 1999; Olander and Vitousek 2000; Sinsabaugh 1994). Enzyme activities were assayed after 1, 7, 14, and 28 days. One unit of activity equals 1 $\mu mol\, h^{-1}\, g^{-1}$ soil-compound mixture. All added enzymes were commercial preparations from Sigma-Aldrich, St. Louis, MO.

Enzyme assays

Activities of AP, BG, and NAG were measured as described in Allison and Vitousek (2004). UR and PPO were assayed similarly, with modifications as follows. The UR assay used 40 mM urea as the substrate, and a nitroprusside/salicylate reaction was used to determine the amount of ammonium produced during the ~4 h assay. 0.5 ml assay supernatant was combined with 2.5 ml of a solution containing 0.354 M sodium salicylate, 0.1 M NaOH, and 1.34 mM sodium nitroprusside. 1.0 ml of 3.91 mM sodium dichloroisocyanurate was then added to develop the color, and the samples were vortexed, incubated for 30 min, and read at 690 nm absorbance. Activities are expressed as nmol $NH_4^+\, h^{-1}\, g^{-1}$ dry soil based on an NH_4^+ standard curve. The PPO assay used 50 mM pyrogallol/50 mM EDTA as the substrate, and the absorbance of the super-

natant from the reaction was determined directly at 460 nm after ~1 h. Activity was converted to μmol pyrogallol $h^{-1} g^{-1}$ dry soil based on the absorbance of product formed by the complete oxidation of a 0.25 mM solution of pyrogallol.

In preliminary experiments, I found that the PPO oxidation product was easily sorbed onto allophane. To correct for these sorption effects, I determined the fraction of product sorbed as a function of product added for each level of mineral addition in separate 1 h incubations. These sorption isotherms showed that soils containing allophane sorbed up to 30% of the PPO oxidation product. Soils with no added minerals sorbed ~15% of the oxidation product, probably reflecting the sorption capacity of minerals already present in the soil. Added ferrihydrite and humic acid did not increase sorption above the 15% background level.

All enzyme activities were corrected to account for dilution by the added compounds. In the irradiation experiment, enzyme activities are reported g^{-1} soil, excluding added compounds. In the enzyme addition experiment, the reported enzyme activities represent the sum of the pre-existing enzyme activity in the soil (g^{-1} soil, excluding added compounds) and the added activity (g^{-1} soil-compound mixture). This calculation results in enzyme activities that are directly comparable across all levels of compound addition at the start of the experiment.

Statistical analyses

Enzyme activities from the control soils of the gamma-irradiation and enzyme addition experiments were fit to the following model:

$$A = A_0 e^{-kt} + b \quad (1)$$

The parameter '*b*' modifies a simple exponential decay model to allow for a non-zero asymptote, which improved the model fit to the data. Parameters and standard errors were estimated using SAS PROC NLIN (SAS Institute 2004). Due to a short supply of synthetic allophane, enzyme assays in the irradiation experiment were not replicated within each sampling date, and therefore statistical differences between control and compound-amended soils could not be assessed.

For the enzyme addition experiment, I used repeated-measures analysis of variance (ANOVA) to test for significant effects ($P < 0.05$) of mineral addition on added enzyme activities. The ANOVAs were run using SAS PROC MIXED (SAS Institute 2004) with a spatial power covariance structure. I tested for differences among levels of mineral addition using post-hoc Tukey tests for multiple comparisons. Initial (day zero) enzyme activities were assumed to be identical for all levels of compound addition, so these data were excluded from the ANOVAs. Enzyme activities were log-transformed where necessary to improve normality.

Results

Irradiation experiment

Following gamma-irradiation, native activity of AP, UR, and PPO declined exponentially at first and then approached an asymptote, suggesting the presence of stabilized enzyme pools (Fig. 1, controls). For BG and NAG, enzyme activities also declined, but the fit to the exponential model was not significant ($P > 0.10$). Decay constants for enzyme activity ranged from -0.032 day^{-1} for BG to -0.628 day^{-1} for PPO (Table 1). The fraction of the initial enzyme activity that was stabilized varied from 35% for UR to 70% for AP (Table 1), although the stabilized fraction of BG and NAG may be less than this range because these activities did not reach a clear asymptote by the end of the incubation.

Added compounds had variable effects on enzyme activities. Although differences could not be statistically tested, added allophane appeared to slow the decay rate of AP (Fig. 1B) and NAG (Fig. 1D). Ferrihydrite addition increased initial UR activity (Fig. 1C), but this activity decayed more rapidly than in the control soil. Initial NAG activity was lower in soil with added ferrihydrite (Fig. 1D), but this activity decayed at a rate similar to NAG in the control soil. Humic acid consistently reduced enzyme activities below

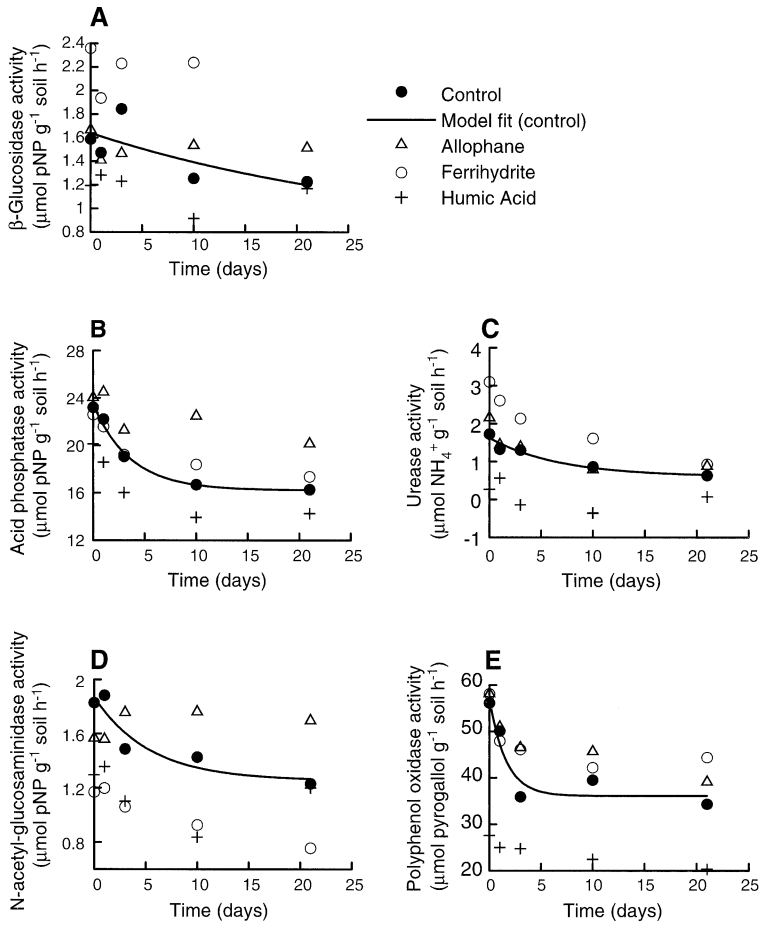

Fig. 1 Native enzyme activities over time in irradiated soils amended with 7.5% allophane, 30% ferrihydrite, 15% humic acid or no amendment (control). (**A**) *β*-glucosidase; (**B**) acid phosphatase; (**C**) urease; (**D**) *N*-acetyl-glucosaminidase; (**E**) polyphenol oxidase. Curve represents fit of first order exponential decay model (see Eq. 1) to control data

control levels, and this effect was particularly pronounced for AP, UR, and PPO (Fig. 1).

Enzyme addition experiment

The range of decay rate constants for added enzymes (– 0.045 to – 0.673 day^{-1}) was similar to the range for native enzymes (– 0.032 to – 0.628 day^{-1}), except for added UR activity which decayed rapidly at a rate of – 8.41 day^{-1} (Table 1). The mean ± SE rate of decay for added enzymes (excluding UR) was – 0.24 ± 0.13 day^{-1} vs. – 0.25 ± 0.10 day^{-1} for native enzymes ($P = 0.95$, NS, *t*-test). The stabilized pools for added enzymes

Table 1 Turnover rate constants (*k*) and stabilized pool sizes (*b*) for enzymes

	P-value (model fit)	*k* ± SE (day^{-1})	*b* ± SE ($\mu mol\, g^{-1}\, h^{-1}$)	*b* (% initial activity)
Irradiation experiment				
β-glucosidase	0.480	– 0.032 ± 0.258	0.752 ± 5.030	47 ± 317
Acid phosphatase	0.011	– 0.277 ± 0.059	16.2 ± 0.4	70 ± 2
Urease	0.053	– 0.149 ± 0.089	0.603 ± 0.202	35 ± 12
N-acetyl-glucosaminidase	0.121	– 0.187 ± 0.161	1.25 ± 0.16	69 ± 9
Polyphenol oxidase	0.096	– 0.628 ± 0.368	36.1 ± 2.9	64 ± 5
Enzyme addition experiment				
β-glucosidase	0.022	– 0.673 ± 0.219	33.2 ± 12.9	13 ± 5
Acid phosphatase	0.025	– 0.147 ± 0.054	35.9 ± 4.0	52 ± 6
Urease	< 0.001	– 8.41 ± 2.58	2.65 ± 0.34	0.23 ± 0.03
N-acetyl-glucosaminidase	0.236	– 0.045 ± 0.079	14.7 ± 31.7	29 ± 63
Polyphenol oxidase	0.0013	– 0.110 ± 0.010	124.6 ± 6.0	39 ± 2

were generally larger than the stabilized pools for native enzymes (compare *b*-values in Table 1), but these latter pools represent a smaller fraction of the initial activity since large amounts of enzyme were added (Table 1, last column).

Allophane at a soil concentration of 3.8% significantly suppressed BG activity by 23–44% (Fig. 2A). However, higher concentrations of allophane did not have significant effects on BG activity. UR activity was only stabilized by allophane at the two highest levels of addition (Fig. 2C). Allophane at all concentrations had a strong positive effect on AP, NAG, and PPO activities (Fig. 2B, D–E). Much of this effect was due to an increase in activity above the added amount during the first week; this increase persisted through the course of the experiment. Rather than simply reducing the decay rate of added enzyme, allophane addition apparently prevented a larger fraction of enzyme from decaying at all. This stabilizing effect was strongest at intermediate levels of allophane addition for AP and PPO (Fig. 2B, E).

Ferrihydrite addition resulted in stabilization of all added enzymes except UR (Fig. 3), although the effect was smaller than for allophane. Increasing amounts of ferrihydrite generally caused greater enzyme stabilization (except for UR), and the stabilization effect was only significant for the one or two highest levels of ferrihydrite addition. As with allophane, an initial spike in enzyme activity that persisted through the incubation accounted for most of the stabilizing effect.

As in the irradiation experiment, there was no evidence from the enzyme addition experiment that humic acids stabilize enzyme activity. The loss of added enzyme activity was rapid in the presence of added humic acid, and it increased with the level of humic acid addition (Fig. 4). At

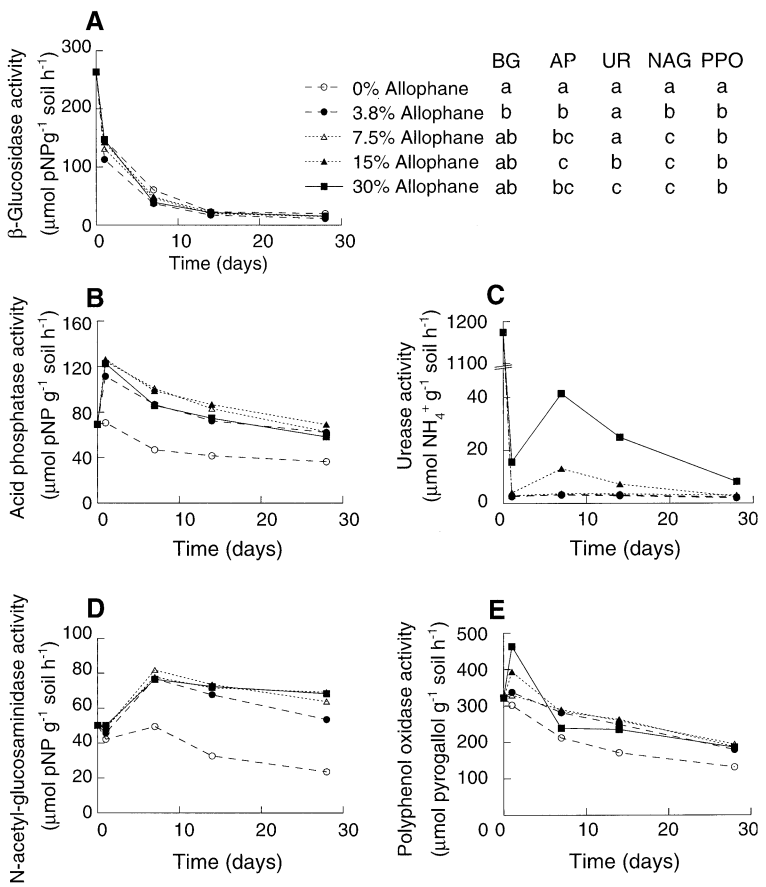

Fig. 2 Enzyme activities in soils with added enzymes from commercial preparations and increasing concentrations of allophane. (**A**) *β*-glucosidase; (**B**) acid phosphatase; (**C**) urease; (**D**) *N*-acetyl-glucosaminidase; (**E**) polyphenol oxidase. Levels of allophane addition with the same letter in the legend are not significantly different ($P \geq 0.05$, post-hoc Tukey test)

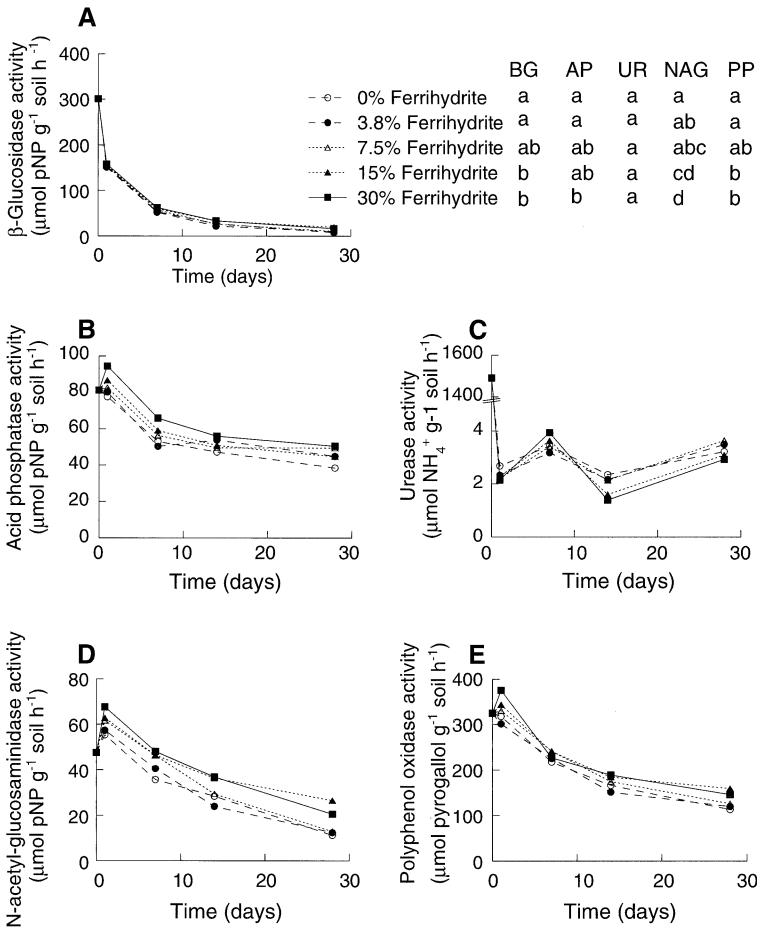

Fig. 3 Enzyme activities in soils with added enzymes from commercial preparations and increasing concentrations of ferrihydrite. (**A**) β-glucosidase; (**B**) acid phosphatase; (**C**) urease; (**D**) *N*-acetyl-glucosaminidase; (**E**) polyphenol oxidase. Levels of ferrihydrite addition with the same letter in the legend are not significantly different ($P \geq 0.05$, post-hoc Tukey test)

the highest levels of humic acid addition, essentially all of the added enzyme activity disappeared by 28 days. The amount of activity remaining was very similar to the native enzyme activity of the soil, before supplementary enzymes were added.

There were significant time × allophane level interactions for all enzymes except BG, and significant time × ferrihydrite level interactions for BG, AP, and PPO (repeated-measures ANOVA, $P < 0.05$). In general, these interactions were less important than the main effect of mineral addition level (compare *F*-values, Table 2). However, for AP and PPO, the interactions occurred because mineral addition at the 30% level had a strong positive effect on enzyme activity early in the incubation, but this effect dropped off over time (Figs. 2B, E, 3B, E). Time × humic acid level interactions were significant for BG, AP, and PPO, but relatively weak compared to the main effects of addition level and time (Table 2).

Discussion

Within the soil, enzymes may be freely dissolved in solution or bound to microbes, organic matter, or mineral surfaces (Burns 1982). The irradiation and enzyme addition experiments confirm that minerals common in Hawaiian soils can stabilize or enhance enzyme activity, but humic acids inhibit enzymes. In general, the positive effect of soil minerals and the inhibitory effect of humic acids applied to enzymes that were already present within the soil (irradiation experiment), or recently added (enzyme addition experiment). However, these effects were stronger and more consistent if the enzymes were recently added. In

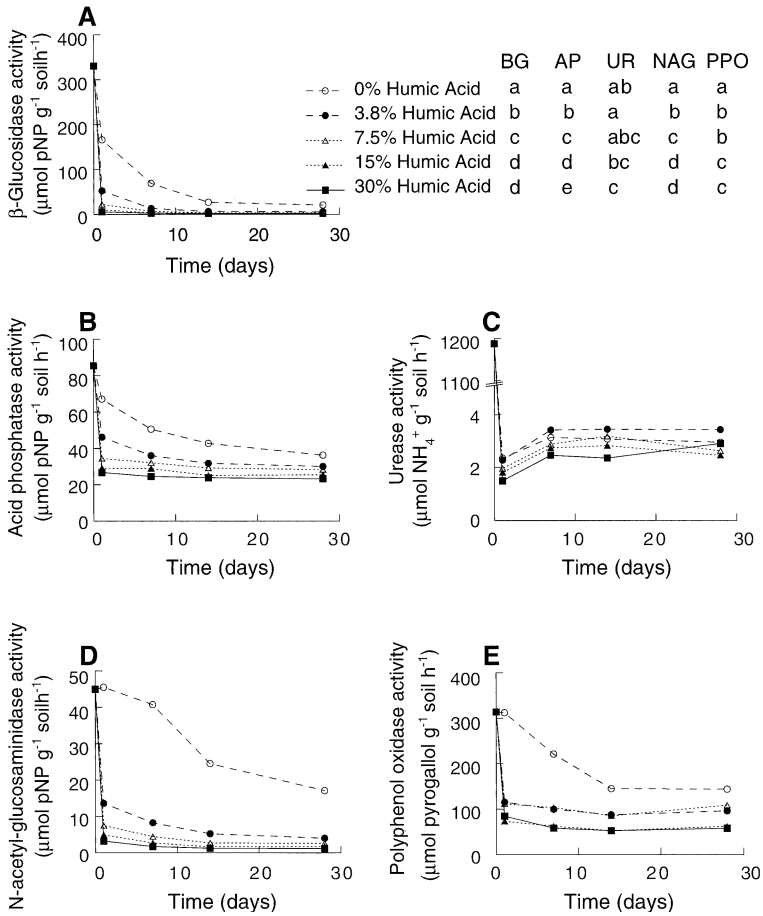

Fig. 4 Enzyme activities in soils with added enzymes from commercial preparations and increasing concentrations of humic acid extracted from Hawaiian soils. (**A**) *β*-glucosidase; (**B**) acid phosphatase; (**C**) urease; (**D**) *N*-acetyl-glucosaminidase; (**E**) polyphenol oxidase. Levels of humic acid addition with the same letter in the legend are not significantly different ($P \geq 0.05$, post-hoc Tukey test)

Table 2 *F*-values for effects of time, compound level, and time × compound level interaction on enzyme activities from repeated-measures ANOVA for the enzyme addition experiment

	Allophane			Ferrihydrite			Humic acid		
	Time	Level	Interaction	Time	Level	Interaction	Time	Level	Interaction
df	(3,15)	(4,5)	(12,15)	(3,15)	(4,5)	(12,15)	(3,15)	(4,5)	(12,15)
β-glucosidase	602.2***	5.3*	1.4^{NS}	750.9***	10.5*	3.9**	258.1***	200.4***	3.9**
Acid phosphatase	509.9***	95.1***	6.3***	385.6***	11.8**	3.6*	146.9***	362.5***	14.5***
Urease	43.4***	166.4***	6.0***	54.3***	0.8^{NS}	1.7^{NS}	66.9***	14.3**	2.0^{NS}
N-acetyl-glucosaminidase	149.6***	171.6***	14.5***	472.1***	25.7**	2.0^{NS}	123.5***	218.9***	2.3^{NS}
Polyphenol oxidase	252.6***	26.5**	10.04***	508.7***	16.4**	2.5*	131.0***	698.0***	14.3***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS = not significant

the irradiation experiment, greater enzyme activity with mineral addition may have been partly due to reduced protease activity, but no such reduction would have occurred in the enzyme addition experiment where greater activities were also observed. Overall, there is good evidence that minerals stabilize or enhance enzyme activities in these soils, although the effect may depend on the type of mineral and enzyme: added BG was inhibited by allophane (Fig. 2A) but slightly enhanced by ferrihydrite (Fig. 3A), while added UR was unaffected by ferrihydrite (Fig. 3C).

The implications of stabilization for enzyme function are unclear. Do minerals increase or decrease actual enzyme activity in the field? In this study, minerals enhanced enzyme activity or allowed a higher concentration of enzyme to persist in the soil. Other studies have also found that minerals stabilize or enhance enzyme activity (Rao et al. 2000; Tietjen and Wetzel 2003), but negative effects on activity are commonly observed (Naidja et al. 2000). However, the enzyme assays conducted in my study and most other studies measure potential activities using soluble substrates, in contrast to field conditions where substrates are commonly insoluble and/or bound to minerals.

Under environmental conditions, stabilized enzymes probably cannot interact efficiently with their substrates, and therefore have low catalytic efficiencies (Allison 2006; Chevallier et al. 2003; Ladd 1985). Studies of enzyme activities in different soil fractions support this idea. For example, Stemmer et al. (1999) found that adding straw increased activities of C-degrading enzymes most dramatically in soil size fractions >200 μm, which contained most of the added straw. Thus the enzymes that were most likely related to straw decomposition were found in the larger, organic-rich size fractions rather than the smaller, mineral-dominated size fractions. Allison and Jastrow (2006) found high potential PPO activity in the clay-sized fraction of grassland soils, despite the slow turnover time of C in that fraction. Thus, the potential activities of enzymes measured in mineral fractions of soil do not necessarily correspond to rapid C turnover in those fractions under field conditions. Even though stabilized enzymes may represent a large fraction of the total pool, reductions in the V_{max} and substrate affinity of stabilized enzymes probably reduce their contribution to ecosystem processes (Gianfreda et al. 1991, 1992). A reduction in efficiency of stabilized enzymes could help explain why many allophane-rich soils have low rates of C turnover relative to other soil types (Rasmussen et al. 2006; Torn et al. 1997).

In many of the mineral-enriched soils, enzyme activity increased substantially above the initial amount added. The mechanism for this increase is unclear, but a likely explanation is that the microbial community produced new enzymes during the incubation. AP, NAG, and PPO activities might have increased because the enzyme proteins added at the start of the experiment provided a labile source of C and N that microbes reallocated to enzyme production (Allison and Vitousek 2005). However, this mechanism cannot account for increased initial activities in mineral-amended soils of the irradiation experiment because microbes were killed (i.e. Fig. 1A, C). This pattern could be explained if the newly added minerals adsorbed existing enzymes in a way that increased potential activity, perhaps by favorably altering the enzyme conformation. Such a mechanism could have also contributed to the increased activity observed in the enzyme addition experiment.

Alternatively, activity enhancement may have occurred due to higher concentrations of enzymes and assay substrates at mineral surfaces as suggested by Tietjen and Wetzel (2003). However, this possibility is unlikely because Tietjen and Wetzel (2003) observed activity increases for BG, and I did not, even though I added the same enzyme preparation. Also, if the enzyme assays are run at substrate saturation (as designed), then higher substrate concentrations should not increase observed enzyme activities. Furthermore, concentration effects can not explain the increase in NAG activity with allophane addition (Fig. 2D) because the increase was delayed until after day one.

In contrast to soil minerals, added humic acid did not stabilize enzyme activity, as predicted. Some of this effect may have resulted from a reduction in soil pH associated with humic acids, although the laboratory assays were conducted under buffered conditions at a constant pH. Humic acids may have formed complexes with added enzymes that altered enzyme conformation or blocked the active site. Sarkar and Burns (1984) also found that incorporation of BG into humic polymers reduced enzyme activity; however, this activity was more stable than the activity of free enzymes once added to soil. In contrast, another study using similar methods with AP-humic polymers did not find greater stability of the polymerized enzyme after addition to soil (Garzillo et al. 1996). Both of these studies

combined phenolic monomers and enzymes to synthesize humic-enzyme polymers, which may have resulted in less occlusion or denaturation of the enzyme than occurs naturally (Vuorinen and Saharinen 1996).

Mineral stabilization and humic acid inhibition of enzyme activities provide strong support for a multi-pool model in which turnover rates vary for enzymes within the soil (Fig. 5). The turnover rates shown in Table 1 for both native and added enzymes correspond to the active pool in Fig. 5, while the *b*-values in Table 1 correspond to the size of the stabilized pool in Fig. 5 which has much slower turnover rates. Enzymes from the active pool may eventually enter the stabilized pool, or interact with humic compounds (Fig. 5). Studies of enzyme dynamics within different soil fractions show that enzyme turnover varies widely and depends on the spatial location of the enzyme within the soil matrix (Allison and Jastrow 2006; Marx et al. 2005). Furthermore, this variation probably occurs at very small spatial scales, with enzyme production, microbial growth, and turnover rates locally elevated near organic substrates. As demonstrated here, the fate and stability of these enzymes ultimately depends on the availability of mineral surfaces near the production site, and whether enzymes are stabilized in newly forming humic polymers or inactivated by interactions with existing humic compounds.

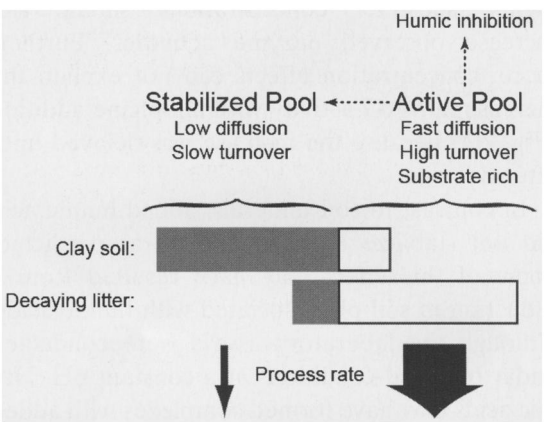

Fig. 5 The multi-pool model of soil enzyme activities. Enzymes in the active pool (open boxes) contribute most to the overall process rate (thickness of solid arrows corresponds to contribution to process rate). Dashed arrows represent movement of enzymes out of the active pool. In mineral-rich soils, only a small fraction of the potential enzyme activity measured in a laboratory assay is present in the active pool. In decaying litter, a larger fraction of the measured potential activity contributes to the process rate. In addition to minerals, organic compounds may also increase the size of the stabilized, low activity pool (gray boxes)

The existence of multiple pools of enzyme activity within soil has long been appreciated (Burns 1982), but has not been well integrated into conceptual models of enzyme function. The addition of organic substrates to soils frequently results in a transient increase in enzyme activity that declines to some background level after the substrate decomposes (Ladd and Paul 1973). In most systems, the bulk of biogeochemical processes probably occurs in microbial 'hotspots' that represent a small fraction of the total soil volume, enzyme activity, and microbial biomass. If a large proportion of the enzyme activity is stabilized on soil particles but has low catalytic efficiency, potential activities in bulk soil may be a poor predictor of nutrient cycling rates. Conversely, low measured enzyme activities may correspond to rapid nutrient cycling in systems where mineral content is low and enzyme production and turnover are high (Fig. 5).

Existing models of enzyme dynamics should consider the strong effects of mineral and humic compounds on enzyme activity and stabilization. The results presented here provide support for a model of enzyme-driven decomposition proposed by Schimel and Weintraub (2003). They suggest that the resource returns to a microbial decomposer must be a non-linear, diminishing function of enzyme production, otherwise microbial population sizes either collapse or explode to infinity. Stabilization and inactivation of enzymes on minerals and humics would result in this type of diminishing return function.

Another well-known model by Sinsabaugh and Moorhead (1994) predicts that microbial allocation to enzyme production depends on the relative availabilities of different nutrients. Consistent with this prediction, many empirical studies have found that organisms produce enzymes to acquire limiting resources (e.g. Allison and Vitousek 2005; Chróst 1991; Sinsabaugh et al. 1993). However, the presence of mineral and humic compounds could obscure these fundamental relationships, and may help to explain why soil enzyme

responses to environmental perturbations (such as CO_2 or nitrogen fertilization) are often ecosystem-specific (Moorhead and Linkins 1997; Sinsabaugh et al. 2003; Waldrop et al. 2004). Across ecosystems, differences in nutrient availability may be confounded with differences in soil mineralogy and humic composition. For example, potential enzyme activity may be high in Andisols because of their ability to sorb and stabilize enzymes. This issue could be addressed by comparing enzyme activity ratios within a soil or substrate type (Sinsabaugh et al. 2002), although preferential stabilization of particular enzymes (e.g. Fig. 2) would complicate such a comparison.

Conclusion

The data presented here suggest that enzyme activity measured in the laboratory represents the sum of active and stabilized enzyme pools. Common soil minerals, such as allophane and ferrihydrite, partially determine the size of the stabilized pool. In contrast, humic acids—among the most abundant organic compounds in soil—strongly inactivate enzyme activity, although enzymes incorporated into humics during polymer synthesis may be more stable. Still, the functional importance of stabilized enzymes remains questionable, and evidence from the literature suggests that the active enzyme pool is more strongly associated with biogeochemical processes. Future studies should attempt to differentiate the contributions of different enzyme pools to ecosystem function (e.g. Stemmer et al. 1999). Alternatively, studies measuring bulk enzyme activities in soil should recognize that a large pool of stabilized enzymes could make changes in the active pool more difficult to detect. This point is particularly relevant for studies that attempt to link enzyme activities with soil quality, mineralization rates, or disturbance because these factors probably correlate more closely with active enzymes than with bulk soil enzymes. Finally, ecosystem models should incorporate multiple pools of enzymes to improve predictions of organic matter decomposition, especially if stabilized enzymes have reduced catalytic efficiency.

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