



Linking microbial community composition to function in a tropical soil

M.P. Waldrop*, T.C. Balser, M.K. Firestone

Department of Environmental Science, Policy, and Management, 151 Hilgard Hall, University of California, Berkeley, CA 94720-3110, USA

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Abstract

If changes in the composition of the soil microbial community alter the physiological capacity of the community then such changes may have ecosystem consequences. We examined the relationships among community composition (PLFA), microbial biomass (CFDE), substrate utilization profiles (BIOLOG), lignocellulose degrading enzyme activities (β -glucosidase, cellobiohydrolase, β -xylosidase, phenol oxidase, peroxidase), and nutrient releasing enzyme activities (phosphatase, sulphatase) in a Tropeptic Haplustol soil. The soils supported a tropical forest and pineapple plantations of varying ages that were at different stages within the management cycle. Conversion from forest to agriculture significantly decreased %C and %N of the soil by 50–55%, microbial biomass by 75%, β -glucosidase by 54%, sulphatase activity by 85%, decreased Ca, Mg, and Mn availability, and produced compositionally and functionally distinct microbial communities. Total enzyme activities were generally correlated with %C, %N, microbial biomass and, occasionally with community composition. We calculated the specific activities of the enzymes assayed (enzyme activity per unit microbial biomass C) in order to normalize activity to the size of the microbial community. Values for enzyme specific activities were more highly correlated with community composition than were total enzyme activities. In addition, BIOLOG was not correlated with community composition or enzyme activities. Enzyme activities and specific activities may provide a useful linkage between microbial community composition and carbon processing. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Microbial community; PLFA; BIOLOG; Enzyme activities

1. Introduction

The aerobic heterotrophic microbial community in soil decomposes organic compounds in order to obtain reducing potential for energy and carbon to build biomass. This universal process clearly underlies sustainable land use and ecosystem carbon balance. Microorganisms have traditionally been regarded as ubiquitous, “everything is everywhere”, which has led to the common assumption that microbial communities are black boxes; passive catalysts for which degradation is controlled by abiotic variables such as nutrient availability, temperature, moisture, pE, and pH. The ‘black-box model’ of soil processes clearly excludes the importance of microbial community composition. Abiotic variables have not, however, been universally successful at predicting degradation rates of carbon compounds as contaminants or naturally occurring molecules (Holden and Firestone, 1997; Zogg et al., 1997). In addition, evidence is beginning to show that the composition of the microbial community affects the degradation rates of soil carbon compounds independent of environmen-

tal variables (Zogg et al., 1997; Degens, 1998, 1999). This has prompted increased research into microbial community composition and community metabolic functions (Zak et al., 1994; Bossio et al., 1998; Fernandez et al., 1999). However, considerable lack of knowledge remains concerning the relationship of community composition and community physiological capacity.

The degradation of macromolecular carbon compounds may be related to community composition because the enzymatic capacity for the initial steps of degradation occurs in a comparatively limited number of microbial populations (Hammel, 1997; Hu and van Bruggen, 1997). Schimel (1995) calls processes based on physiological capacities of limited distribution ‘narrow’ processes. Several groups have reported the effect of a change in microbial community composition on process rates (Schimel, 1995; Berg et al., 1995; Andrén et al., 1999; Zogg et al., 1997; Balser et al., 2001). Oxidative enzymes, such as phenol oxidase and peroxidase, are produced by a relatively restricted group of microorganisms (Hammel, 1997), but are not limited to fungi (Falcon et al., 1995). Cellulases are probably produced by more organisms than ligninases, but their relationship to shifts in community composition in

* Corresponding author.

natural environments is still poorly understood. Macromolecular carbon degradation is a synergistic result of many populations interacting in consortia. In addition, fungal interactions with bacteria can have profound effects on lignocellulase activities (Lang et al., 1997). Thus, a broad approach that considers the components and activities of the entire soil community may be productive. The analysis of microbial phospholipid fatty acids (PLFAs) is a broad measure of microbial community composition. PLFA analysis can be used to estimate the relative sizes of fungal, actinomycete, anaerobe, Gram + and Gram – communities. We attempted to link PLFA biomarker data with two functional measures, lignocellulase activities and substrate utilization capacities (BIOLOG), of several soil microbial communities under changing land use conditions.

An understanding of the relationships between the composition of the microbial community and its physiological capacity is particularly important in agroecosystems. In these ecosystems, frequent soil disturbance, carbon and nutrient alterations, and changes in the water and light regime can alter the composition of the microbial community (Kennedy and Smith, 1995; Bossio et al., 1998). We analyzed soils from five tropical forest sites surrounding pineapple plantations of differing ages and stages within the management cycle. Our objectives were to assess the composition of the soil microbial community and its functional capabilities across a conversion in land use from tropical forest to cultivated soils and to answer two questions. In what ways do these soils differ in microbial biomass, community composition, and physiological capabilities? Can lignocellulase activities be related to changes in community composition which occur with changing land use?

2. Materials and methods

2.1. Field sites

The research was conducted in Tahiti (17°30'S, 149°50'W) on the Society Islands of French Polynesia. The dominant plant species in our forest sites were *Hibiscus tillaceus*, *Nephrolepis* and *Gleichenia linearis*, *Psidium guajava*, *Pistache*, and *Eugenia cumini*. After clearcutting the forest, the forest litter was burned and the plantations planted within one year. The same owner used similar management practices on each plantation. Thirty thousand pineapple plants (*Bromilacea ananas comelsus* var *quentii*) per hectare were grown for three to four years after which time the plants became unproductive and were chipped and incorporated into the surface soil. Each plant was given 80 g fertilizer per year (30:10:30:10 — N:P:K:S). Precipitation is 1728 mm annually, mostly falling in winter, and mean annual temperature is 26°C. The soil is a Tropeptic Haplustol and the soil texture is a silt loam to sandy loam with <5% clay.

Five tropical forest sites were selected randomly from the perimeter of the pineapple plantations. One 30 m transect was placed in each forest site. Four plantations were selected that differed in age or stage within the management cycle. The youngest was a six-year old plantation with three-year old pineapple plants. The second, a nine-year old plantation was bare: the previous rotation of pineapple plant biomass was chipped and incorporated into the soil within the preceding three months. The third, a twelve-year old plantation, was at the same stage within the management cycle as the nine-year old plantation. The fourth, a fourteen-year old plantation had three-year old pineapple plants on it. Thus, the six- and nine-year old plantations were considered the 'young' treatment. The twelve- and fourteen-year old plantations were considered as the 'old' treatment. The six- and fourteen-year old plantations were considered as the 'mature plant' treatment and the nine- and twelve-year old plantations were considered the 'bare' treatment. Three 30-m transects were established from the center of each plantation along a randomly determined compass heading. Every three meters along each transect, soil cores (12.5 cm long × 4.5 cm diameter) were driven into the soil with a mallet. The following day, cores were pulled from the soil, wrapped in plastic, and placed in a cooler at ~4°C. Soils were transported to the laboratory within 24 h of sampling. All soils were sampled in July 1998. Three cores were randomly selected from each transect, bulked, and homogenized by mixing and removing roots and coarse organic material. Analyses were performed on three composited samples per transect, except where indicated ($n = 15$ for forest; $n = 9$ per plantation).

2.2. Enzyme activities

The enzymes assayed were β -1,4-glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91), β -xylosidase (EC 3.2.1.37), phenol oxidase (EC 1.10.3.2), peroxidase (EC 1.11.1.7) phosphomonoesterase (EC 3.1.3.2) and sulphatase (EC 3.1.6.1). The substrates for the β -glucosidase, β -xylosidase, cellobiohydrolase, phosphatase, and sulphatase assays were *p*-nitrophenol (*p*NP) β -D-glucopyranoside, *p*NP- β -xylopyranoside, *p*NP-cellobioside, *p*NP-phosphate, bis-*p*NP-phosphate, and *p*NP-sulphate, respectively. Phenol oxidase substrate was 10 mM L-dihydroxyphenylalanine (DOPA). Peroxidase substrate was 10 mM L-DOPA solution and 0.3% H₂O₂. All substrates were made in 50 mM pH 5.0 acetate buffer. Five grams of soil were added to 50 ml 50 mM acetate buffer solution (pH 5.0), briefly shaken by hand and stirred on a stir plate for 30 s. Fifty μ l of the mixture was added to each lane of a 96-well microplate that contained 150 μ l of 50 mM *p*NP-substrate solution or acetate buffer (sample control). There were five analytical replicates and eight sample controls of each extract. Plates were incubated at 27°C for 2 h after which time 50 μ l of the solution in each well was transferred to another 96-well plate containing 50 μ l of 1.0 N

Table 1
Effect of land use treatments on physical, chemical, and biological properties of soils

Variable	Forest	Young plantation	Old plantation	Bare	Mature plant
θ_m^\dagger	0.32 ^{ax}	0.29 ^b	0.27 ^c	0.28 ^y	0.28 ^y
pH [‡]	4.57 ^{ax}	4.29 ^a	3.97 ^b	4.21 ^y	4.00 ^y
%C [†]	7.81 ^{ax}	3.52 ^b	3.60 ^b	3.52 ^y	3.60 ^y
%N [†]	0.40 ^{ax}	0.20 ^b	0.19 ^b	0.17 ^y	0.21 ^z
Ca [†] ($\mu\text{g } 10 \text{ g}^{-1}$)	7.0 ^{ax}	1.0 ^b	0.4 ^c	0.7 ^y	0.8 ^y
K [†] ($\mu\text{g } 10 \text{ g}^{-1}$)	1.2 ^{ax}	0.6 ^a	0.6 ^a	0.8 ^x	0.3 ^y
Mg [†] ($\mu\text{g } 10 \text{ g}^{-1}$)	5.3 ^{ax}	0.3 ^b	0.1 ^c	0.2 ^y	0.1 ^z
Mn [†] ($\mu\text{g } 10 \text{ g}^{-1}$)	6.5 ^{ax}	2.7 ^b	0.9 ^c	2.0 ^y	1.6 ^y
P [†] ($\mu\text{g } 100 \text{ g}^{-1}$)	4.8 ^{ax}	8.8 ^a	3.8 ^b	4.3 ^x	9.7 ^x
S [†] ($\mu\text{g } 100 \text{ g}^{-1}$)	1.3 ^{ax}	1.8 ^a	2.3 ^a	1.7 ^x	2.6 ^y
Zn [†] ($\mu\text{g } 10 \text{ g}^{-1}$)	4.3 ^{ax}	9.8 ^a	5.6 ^a	9.1 ^x	6.8 ^x
Biomass [‡] ($\mu\text{g } \text{g}^{-1}$)	855 ^{ax}	222 ^b	209 ^b	193 ^y	238 ^y
Labile C [‡] ($\mu\text{g } \text{g}^{-1}$)	258 ^{ax}	198 ^a	188 ^b	171 ^y	216 ^x

θ_m is the mass water content of soil. The 'Bare' treatment recently had mature plant biomass chipped and incorporated into the soil. The 'Mature plant' treatment had mature pineapple plants growing on the soil. Letters denote differences among treatments (a,b,c among forest, young, and old; x,y,z among forest, bare, and mature plant). Values are means ([†] $n = 3$ for forest and 6–9 for plantation treatments; [‡] $n = 14$ –15 for forest and plantation treatments).

NaOH to terminate the reaction. In the case of phenol oxidase and peroxidase, however, aliquots were transferred to empty wells. Absorbance was measured at 410 nm for *p*NP-substrates and at 469 nm for L-DOPA assays using a Spectramax plus spectrophotometer (Molecular Devices). Peroxidase activity was calculated as the difference between samples reacted with and without H₂O₂ (Sinsabaugh et al., 1993). Units for phenol oxidase and peroxidase are in absorbance units h⁻¹ g⁻¹ dry soil. All other enzyme activities were expressed as μmol substrate converted h⁻¹ g⁻¹ dry soil. The specific activities of the enzymes were calculated by dividing total enzyme activities by the microbial biomass C determined by CFDE.

2.3. Microbial community composition

Phospholipid fatty acid analysis was conducted on 5 g samples of freeze dried soil using the procedure by White and Ringelberg (1998). Extracted phospholipid samples were analyzed using a Hewlett Packard 6890 Gas Chromatograph with a 25 m \times 0.2 mm \times 0.33 μm Ultra 2 (5%-phenyl)-methylpolysiloxane column (Hewlett Packard) using hydrogen as the carrier, nitrogen as the make up gas, and air to support the flame. The GC analyzed a 1 μl injection with a 1:100 split, at an initial temperature of 170°C, ramped to 260°C at 2°C min⁻¹ at a constant flow rate of 0.4 ml per min, with a rapid increase to 310°C for 2 min at the end of each run to bake out the column. Run time was approximately 27 min per sample. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE).

2.4. Substrate utilization patterns

Substrate utilization patterns were measured using the BIOLOG[™] Gram-negative microtiter plates (BIOLOG, Inc.). Methods were similar to those of Bossio and Scow (1995). Twenty gram samples of field moist soil were

shaken for 1 h in 200 ml 0.7% m/m NaCl and aliquots from a 10⁻³ dilution were pipetted into plates using an 8-channel micropipetter set to 150 μl . Plates were incubated at 28°C and read on a BIOLOG[™] Microplate Reader every 12 h. Color development in the control well (due to utilization of background dissolved organic carbon) was subtracted from absorbance readings in all other wells. Negative values were set to zero. A time point was chosen based on its average well color development (AWCD) to analyze for each plate (Garland, 1996). Time points chosen had AWCD values between 0.75 and 1.0. Prior to statistical analysis, individual well absorbance values were normalized by total plate color to account for possible differences in inoculation densities between samples. These processed data were used for principal components analysis.

2.5. Microbial biomass, nutrients, and pH

Microbial biomass C and N were measured using the chloroform fumigation and direct extraction (CFDE) technique (Vance et al., 1987). A conversion factor of 2.64 was used to convert extracted C to biomass C. Available nutrients were determined using the Mehlich 3 extraction method (Mehlich, 1984). Extracts were then analyzed for P, S, Ca, Mg, and Mn on an Inductively Coupled Plasma Spectrophotometer (Thermo Jarrell Ash). Total C and N of oven dried soils were measured by combustion on a Carlo-Erba NA 2100 carbon and nitrogen analyzer. For pH measurements, soil samples were mixed with an adequate amount of deionized water to create a flowing paste. The paste was allowed to equilibrate for several minutes and the pH of the paste was determined using an Accumet Basic pH meter (Fischer Scientific).

2.6. Statistics

Analysis of Variance (ANOVA) was performed using Super ANOVA software (Abacus Concepts). Tukey–Kramer

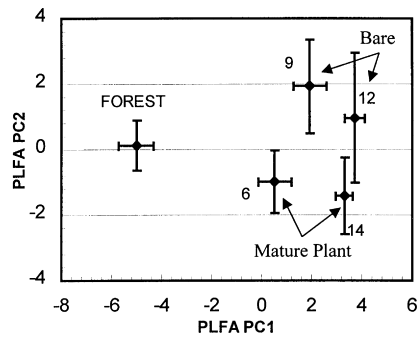


Fig. 1. Microbial community composition among land-use treatments. Nine and twelve year-old plantations are 'bare' treatments: pineapple biomass had been chipped and incorporated into the soil before the next rotation. The six- and fourteen-year-old plantations had mature three-year-old pineapple plants on them. Error bars are one SE ($n = 14$ for the forest, $n = 9$ for the six, nine, and 14-year old plantations, and $n = 4$ for the 12-year old plantation).

highly significant difference (HSD) tests and Scheffe's S tests (in case of nonparametric data) were performed as post-hoc tests. Principal Components Analysis (PCA) was performed using JMP software (SAS Institute). The first and second principal components (PC1 and PC2, respectively) were used for

further correlation analysis. The Spearman's Rho correlation analysis was used in case of nonparametric data. All effects noted were significant at the $P < 0.05$ level.

3. Results

3.1. Effect of treatments on pH, nutrients, and microbial biomass

Conversion of tropical forest to pineapple plantation decreased the pH of the soil, %C, %N, and the availability of all nutrients except K, S, and Zn. As the plantations aged, the pH and the availability of Ca, Mg, Mn, and P decreased (Table 1). The bare soil plantations into which plant biomass had recently been tilled showed decreased %N and available S and increased the availability of Mn and Mg compared to the mature plantations (Table 1). The C:N ratios were not significantly different among all the soils (data not shown). Microbial biomass was four times larger in the forest than in the plantation treatments, but there were no differences in microbial biomass among the cultivated soils. Labile C was highest in the forest and lowest in the old and recently tilled plantations (Table 1).

Table 2
Effect of land use treatments on PLFA content (mol%) of soils

PLFA marker	Forest	Young plantation	Old plantation	Bare	Mature plant
<i>Saturated</i>					
14:0	0.85 ^{ax}	1.15 ^b	1.17 ^b	1.20 ^y	1.12 ^y
15:0	0.84 ^{ax}	1.06 ^b	0.99 ^b	1.12 ^y	0.97 ^x
16:0	13.23 ^{ax}	14.46 ^a	13.24 ^a	14.14 ^x	13.78 ^x
18:0	4.31 ^{ax}	5.51 ^b	6.20 ^c	5.50 ^y	6.01 ^z
<i>Gram +</i>					
i15:0	13.90 ^{ax}	10.90 ^b	9.28 ^c	9.67 ^y	10.55 ^y
a15:0	4.87 ^{ax}	4.02 ^b	4.04 ^b	3.81 ^y	4.17 ^y
i16:0	4.59 ^{ax}	5.07 ^b	5.78 ^c	5.65 ^y	5.20 ^z
16:0 10Me	7.33 ^{ax}	6.27 ^b	4.52 ^c	5.56 ^y	6.13 ^z
a17:0	2.57 ^{ax}	2.15 ^b	2.20 ^b	2.02 ^y	2.28 ^z
<i>Gram -</i>					
16:1c7/15:0 2OH	2.40 ^{abx}	2.65 ^a	2.04 ^b	2.57 ^x	2.25 ^x
i17:1/a17:1	0.72 ^{ax}	2.05 ^b	2.12 ^b	1.94 ^y	2.17 ^y
18:1 ω 7c/ ω 9t/ ω 12t/ ω 9	5.25 ^{ax}	3.77 ^b	2.45 ^c	3.59 ^y	2.92 ^z
15:0 3OH	1.48 ^{ax}	1.16 ^a	1.34 ^a	1.03 ^y	1.38 ^x
16:1 ω 5c	3.02 ^{ax}	3.35 ^b	3.07 ^a	3.36 ^y	3.13 ^x
16:1 2OH	2.04 ^{ax}	3.15 ^b	5.18 ^c	3.91 ^y	4.12 ^y
cy17:0	1.51 ^{ax}	1.97 ^b	1.92 ^b	1.97 ^y	1.93 ^y
cy19:0 ω 8c	13.05 ^{ax}	10.06 ^b	11.71 ^a	10.64 ^y	10.88 ^y
18:1 ω 5c	1.40 ^{ax}	0.46 ^b	0.00 ^c	0.04 ^y	0.41 ^y
18:1 ω 9c	4.34 ^{ax}	4.63 ^a	3.73 ^b	4.85 ^y	3.81 ^z
<i>Fungi</i>					
18:2 ω 6/a18:0	1.26 ^{ax}	1.90 ^b	1.45 ^a	2.04 ^y	1.48 ^x
<i>Actinomycete</i>					
18:0 10Me	0.90 ^{ax}	1.85 ^a	2.63 ^b	1.92 ^x	2.37 ^y

Only PLFAs > 1 mol% are shown. Values are means ($n = 14$ for forest treatment, 13–18 for plantation treatments). Letters denote differences among treatments (a,b,c among forest, young, and old; x,y,z among forest, bare, and mature plant).

Table 3
Effect of land use treatments on total enzyme activities of soils

Variable	Forest	Young plantation	Old plantation	Bare	Mature plant
β -glucosidase	144 ^{abx}	194 ^a	66 ^b	99 ^x	151 ^x
β -xylosidase	44 ^{ax}	28 ^a	32 ^a	28 ^x	32 ^x
Cellobiohydrolase	22 ^{ax}	22 ^a	16 ^a	16 ^x	21 ^x
Phenol oxidase \times 100	79 ^{ax}	103 ^{ab}	102 ^b	124 ^y	77 ^x
Peroxidase \times 100	15 ^{ax}	41 ^a	66 ^b	67 ^y	40 ^x
Phosphatase	302 ^{ax}	706 ^b	578 ^b	711 ^y	558 ^y
Sulphatase	132 ^{ax}	23 ^b	16 ^b	20 ^y	18 ^y

Activity units for enzymes except phenol oxidase and peroxidase are $\mu\text{mol h}^{-1} \text{g}^{-1}$ oven dry soil. Phenol oxidase and peroxidase units are absorbance units $\text{h}^{-1} \text{g}^{-1}$ oven dry soil. Values are means ($n = 13\text{--}18$). Letters denote differences among treatments (a,b,c among forest, young, and old; x,y,z among forest, bare, and mature plant).

3.2. Effect of treatments on microbial community composition

The first principal component (PC1) of the PLFA (mol%) values differed between the forest treatment and all the plantation treatments (Fig. 1). Within the plantation treatments PLFA PC1 of the young mature plantation (six-year) differed from the two old plantations (12- and 14-year) and PC1 of the young bare plantation (nine-year) differed from the old bare plantation (12-year). PC1 was not significantly different between the two young plantations or between the two old plantations. PLFA PC2 differentiated the bare from the mature plant treatments (Fig. 1). PC1 of the PLFA data accounted for 28% of the variability and the second principal component (PC2) explained an additional 12%.

Field treatments had different amounts of individual fatty acids and fatty acid ratios (Table 2). The saturated fatty acids, indicative of bacteria in general, increased in all plantations compared to the forest soils and increased in the bare soils compared to the soils with mature plants (except for 16:0). The Gram + bacteria markers, predominantly composed of branched fatty acids, showed the opposite trend compared to the saturated fatty acids: they decreased in the plantation soils and were lower in bare soils compared to soils with mature plants (except 16:0). The Gram – bacteria markers (monoenoic, cyclopropane, and hydroxy-fatty acids; Wilkinson, 1988) showed no discernable pattern in response to management treatments (Table 2). The

biomarker indicative of fungi, polyenoic, unsaturated fatty acids (Federle et al., 1986) and the actinomycete marker, 18:0 10Me (Vestal and White, 1989), increased in the plantation soils compared to the forest. Within the plantations, the fungal marker increased and the actinomycete marker decreased in the bare soils relative to the soils with mature plants (Table 2).

3.3. Effect of land use treatments on soil enzyme activities

The hydrolytic C-degrading enzyme β -glucosidase had lower activity in the old plantation soils compared to the young plantation soils but the forest treatment was not significantly different from either of the plantation treatments (Table 3). β -xylosidase and cellobiohydrolase activities did not differ among the treatments. The sampling time within the management cycle (bare vs. mature plant) had no effect on any of the hydrolytic enzymes. Total cellobiohydrolase and β -xylosidase activities were significantly correlated with %C and %N ($P < 0.05$) but not significantly correlated with microbial biomass. β -glucosidase was not significantly correlated with %C, %N, or microbial biomass. Total β -xylosidase activity was also correlated with available S ($P < 0.05$).

The total activity of peroxidase but not phenol oxidase was higher in the old plantation soils compared to the forest and younger plantation soils (Table 3). Total phenol oxidase and peroxidase activities were also affected by the sampling

Table 4
Effect of land use treatments on specific activity of enzymes

Variable	Forest	Young plantation	Old plantation	Bare	Mature plant
β -glucosidase	0.20 ^{ax}	1.35 ^c	0.44 ^b	0.38 ^x	1.01 ^y
β -xylosidase	0.09 ^{ax}	0.23 ^b	0.18 ^b	0.20 ^x	0.21 ^x
Cellobiohydrolase	0.03 ^{ax}	0.12 ^c	0.08 ^b	0.09 ^y	0.11 ^y
Phenol oxidase \times 100	0.13 ^{ax}	0.61 ^{ab}	0.61 ^b	0.65 ^y	0.57 ^{xy}
Peroxidase \times 100	0.02 ^{ax}	0.28 ^b	0.44 ^b	0.40 ^y	0.34 ^y
Phosphatase	0.44 ^{ax}	3.71 ^b	3.56 ^b	3.76 ^y	3.47 ^y
Sulphatase	0.06 ^{ax}	0.25 ^a	0.11 ^a	0.11 ^x	0.10 ^x

Activity units for enzymes except phenol oxidase and peroxidase are $\mu\text{mol h}^{-1} \text{g}^{-1}$ microbial biomass C. Phenol oxidase and peroxidase units are absorbance units $\text{h}^{-1} \text{g}^{-1}$ microbial biomass C. Microbial biomass C was measured by CFDE (Table 1). Values are means ($n = 11\text{--}16$). Letters denote differences among treatments (a,b,c among forest, young, and old; x,y,z among forest, bare, and mature plant).

Table 5
Correlation coefficients between total and specific enzyme activities and microbial community composition (PLFA PC1)

	PLFA PC1	
	Total activity	Specific activity
β -glucosidase	*	< 0.1
β -xylosidase	NS	< 0.1
Cellobiohydrolase	NS	***
Phenol oxidase	NS	***
Peroxidase	**	**
Phosphatase	**	***
Sulphatase	***	*

Symbols *, **, and *** represent $P < 0.05$, $P < 0.005$, and $P < 0.0001$, respectively ($n = 41-46$).

time in the management cycle. Both were higher in the bare soils compared to the mature plant soils (Table 3). Activities of both oxidative enzymes were negatively correlated with %C, %N ($P < 0.05$); peroxidase activity was also significantly correlated with microbial biomass ($P < 0.005$).

Total phosphatase activity was higher and sulphatase activity was lower in the plantation soils compared to the forest soils. The sampling time in the management cycle did not affect the total activity of these enzymes (Table 3). Phosphatase was negatively correlated with microbial biomass ($P < 0.005$) and not correlated with available P, %C, or %N. Sulphatase activity was not correlated with %C and %N, positively correlated with microbial biomass ($P < 0.0001$) and negatively correlated with available S ($P < 0.05$).

In order to normalize enzyme activities across differences in standing microbial biomass, the total enzyme activity data were transformed to specific activity by dividing the

Table 6
Significant correlation coefficients (r) between specific activities of enzymes and the mol% of individual PLFAs

	GLUC	XYLO	CBH	PO	PER	PHOS	SULF
<i>Saturated</i>							
14:0	0.48*		0.42*	0.56**	0.50**	0.55**	
15:0	0.35*		0.44*	0.34*	0.46**	0.62**	
17:0			0.39*	0.37*			
18:0				0.54**	0.34*	0.45*	
<i>Gram +</i>							
i15:0			-0.61**	-0.71***	-0.50**	-0.64***	0.43*
a15:0			-0.57**	-0.49**	-0.39*	-0.52**	0.45*
i16:0			0.42*	0.42*	0.49**	0.56**	-0.38*
16:0 10Me			-0.54**	-0.68***	-0.34*	-0.47*	0.57**
cy17:0	0.55**			0.58**	0.57**	0.62**	
a17:0			-0.55**	-0.62***	-0.42*	-0.53**	0.52**
17:0 10Me		0.36*	0.56**	0.52**	0.45*	0.60**	
i18:0			0.39*	0.46**	0.33*	0.45*	
i19:0	-0.45*					-0.38*	
<i>Gram-</i>							
11:0 3OH	0.40*						
i15:1AT5	-0.46*		-0.66***	-0.69***	-0.63***	-0.78***	
i15:1G	-0.42*		-0.70***	-0.61***	-0.48**	-0.68***	0.38*
16:1 ω 5c	0.39*						
16:1 ω 11c			-0.50**	-0.57**	-0.37*	-0.52**	
16:1 2OH			0.52**	0.58*	0.50**	0.65***	-0.37*
17:0 3OH			0.50**	0.52**	0.45*	0.65***	
17:1 ω 8c			-0.41*	-0.33*		0.45*	
i17:1I; a17:1B	0.53**		0.59**	0.64***	0.50**	0.59**	
i18:1 H			0.41*			0.39*	
18:1 ω 5c			-0.54**	-0.56**	-0.44*	-0.66***	0.40*
18:1 w7c,9t,12t			-0.53**	-0.65***	-0.54**	-0.63***	0.38*
18:1 ω 9c					-0.39*		
cy19:0 ω 8c	-0.49**				-0.9*		
<i>Fungi</i>							
18:2 ω 6			0.37*	0.36*			
<i>Actinomycetes</i>							
18:0 10Me				0.51**	0.39*	0.35*	

GLU β -glucosidase; CBH is cellobiohydrolase; XYLO is β -xylosidase; PO is Phenol oxidase; PER is Peroxidase; PHOS is phosphatase activity; SULF is sulphatase activity; symbols *, **, and *** represent $P < 0.05$, $P < 0.005$, and $P < 0.0001$, respectively ($n = 41-46$).

enzyme activity by the microbial biomass C as determined by the CFDE method. The specific activities of all three of the carbon-degrading hydrolytic enzymes (β -glucosidase, β -xylosidase, and cellobiohydrolase) showed similar patterns with the highest values in the young plantations, intermediate in the old plantations, and lowest in the forest treatment (Table 4). The bare treatment with recently incorporated plant debris, decreased the specific activity of β -glucosidase compared to the mature plant treatment, but was not significantly different in specific activity of either β -xylosidase or cellobiohydrolase. The specific activities of phenol oxidase and peroxidase were lower in the forest than in plantations and were largely unaffected by the incorporation of plant material in the bare treatment. The specific activity of phosphatase increased in the plantation soils compared to the forest and the specific activity of sulphatase did not differ among any of the treatments.

We created an aggregate measure of the potential physiological activity of the microbial community by using PCA. This was performed with all assayed enzymes as input variables. Total enzyme activities were normalized to a relative value for the entire data set before they were entered into PCA. PC1 accounted for 36% of the variability in the data while the second principal component accounted for an additional 26%. PC1 of the enzyme activities was correlated with %C ($P < 0.0001$), %N ($P < 0.0001$) and available P ($P < 0.05$), Ca ($P < 0.05$), Mg ($P < 0.05$), and Mn ($P < 0.05$).

The 95-variable BIOLOG data were analyzed by PCA. PC1 and PC2 accounted for 28 and 12% of the variability, respectively. BIOLOG PC1 did not differ between the forest and plantation treatments and only differed between the young-bare and old-mature plant soils. BIOLOG PC2 did not differ among any of the soils (data not shown). Neither BIOLOG PC1 nor PC2 correlated with %C, %N, microbial biomass C, or available nutrients. BIOLOG data were not available for the 12-year old plantation.

3.4. Relating measures of function to composition

We determined if differences in the composition of the microbial community (measured as the first principal component of the PLFA data) were correlated with differences in the physiological capacity of the microbial community (measured either as individual enzyme activities or BIOLOG PC1). No enzyme activities were correlated with PLFA PC2 ($P < 0.05$). Total cellobiohydrolase, β -xylosidase, and phenol oxidase activities were not correlated with community composition. Community composition was correlated with β -glucosidase ($P < 0.05$), peroxidase ($P < 0.005$), phosphatase ($P < 0.005$) and sulphatase activities ($P < 0.0001$) (Table 5). However, peroxidase, phosphatase and sulphatase activities were also highly correlated to microbial biomass, which was itself highly correlated to community composition ($P < 0.0001$).

After transforming total enzyme activity to specific activ-

ity, PLFA patterns were consistently correlated with enzyme activities per unit microbial biomass. PLFA PC1, but not PLFA 2, was correlated with the specific activities of β -glucosidase ($P < 0.1$), β -xylosidase ($P < 0.1$), cellobiohydrolase ($P < 0.0001$), phenol oxidase ($P < 0.0001$), peroxidase ($P < 0.005$), phosphatase ($P < 0.0001$) and sulphatase ($P < 0.05$) (Table 5). PLFA PC1 and PLFA PC2 were not correlated with BIOLOG PC1 or BIOLOG PC2.

We then determined whether mol% values of individual PLFAs were correlated with the specific activities of the enzymes (Table 6). The specific activity of β -xylosidase was correlated to one fatty acid. The specific activities of β -glucosidase and sulphatase were correlated with 9–10 primarily Gram – fatty acids ($P < 0.05$) (Table 6). The specific activities of cellobiohydrolase, phenol oxidase, peroxidase, and phosphatase, however, were correlated with a large number (22–23) of fatty acids representing Gram-positive and Gram-negative bacteria, fungi and actinomycetes. The fungal biomarker (18:2 ω 6) was positively correlated with the specific activity of cellobiohydrolase ($P < 0.05$) and phenol oxidase ($P < 0.05$). The actinomycete biomarker (18:0 10Me) was positively correlated with the specific activity of peroxidase ($P < 0.05$), phenol oxidase ($P < 0.005$), and phosphatase ($P < 0.05$).

4. Discussion

The conversion of a tropical forest to pineapple plantation agriculture had a clear effect on biotic and abiotic properties of the soil. Decreases in available nutrients due to cultivation are consistent with the decrease of soil organic carbon. Soils from bare plantations had less labile C, total N, and available S in the surface 12 cm soil than the plantations that had mature 3-year old pineapple plants on them (Table 2). Cultivation also shifted the distribution of Gram + and Gram-bacteria, fungi, and actinomycetes. According to PLFA biomarkers, conversion from a forest to a plantation increased the relative amount of fungi and actinomycetes, and decreased the relative amount of Gram + bacterial biomarkers. The differences between forest and plantation treatments were generally larger than those among the cultivated soils. The sampling time within the management cycle (bare vs. mature plant) altered community composition (PLFA PC1 and PC2), but had no consistent effect on biomarkers for bacteria. The relative size of the fungal biomarker increased in the plantations compared to forest, and was higher in the bare soils compared to the mature plant soils. Since the total microbial biomass tended to be higher in the mature plant soil compared to the bare soil, we cannot determine if the actual amount of fungi decreased in this treatment.

Changes in community composition can affect the physiological capacity of the microbial community. In our study, BIOLOG was not able to distinguish the effects of treatments on the “functional potential” of the microbial community. In contrast to results from PLFA, biomass,

and enzyme assays, BIOLOG was unable to distinguish the forest treatment from the plantation treatments. Differences in BIOLOG and PLFA results have been seen by others (Buyer and Drinkwater, 1997). Differences may result in part due to the fact that BIOLOG selects for only a portion of the microbial community (Smalla et al., 1998) while PLFA analysis includes the whole microbial community, regardless of activity. Enzyme activities may provide functional measures with more immediate ecological meaning and hence be more clearly related to shifts in microbial community composition. Greater focus on relationships between community composition and function may improve our understanding of soil carbon transformations (Buyer and Drinkwater, 1997; Degens, 1998).

For the past several decades, enzyme activities have been used in a variety of ways to assess issues of environmental quality. They have been tested as indices of site fertility, soil quality, soil productivity, pollution effects, and nutrient cycling potential (Dick and Tabatabai, 1992; Dick, 1994a; Tate, 1995). There has been no consensus concerning which enzymes relate best to site fertility, although phosphatase, cellulase, and groups of enzymes have been correlated with site productivity in different systems (Dick and Tabatabai, 1992). In our analysis we found that all enzymes, except β -glucosidase, phosphatase and sulphatase, were related to the N content of the soil. However, the total N content of the soil is not a good measure of N availability. No single enzyme activities were correlated with available P, Ca, Mg, Mn, K, or Zn (data not shown). Available S was correlated to β -xylosidase and sulphatase activity. The PC1 of all enzyme activities was correlated with available P, Ca, Mg, and Mn, %N, and %C and typically improved the correlation over single enzyme variables. This supports the idea that an aggregate of enzyme activity may be better correlated with soil fertility than single enzymes (Dick and Tabatabai, 1992).

Researchers have found that intensive agriculture in temperate regions appears to depress soil enzyme activities, while cultivated soils in tropical regions that receive substantial organic input have maintained similar or higher enzymatic levels compared to uncultivated soils (Dick et al., 1994b). In the Tahitian plantations studied here, plant biomass is incorporated into the soil every three to four years, and most enzyme activities did not decline in these soils over fourteen years of cultivation. Total β -glucosidase and sulphatase, however, had lower activity in the plantations compared to the forest, and β -glucosidase decreased as the plantation aged. Sulphatase may have been depressed by the use of fertilizer and reduced β -glucosidase activity may have simply resulted from a decrease in microbial biomass.

Total β -glucosidase, phosphatase, sulphatase, and peroxidase activities were correlated with community composition (Table 5). Other researchers have shown that variation in the PLFA pattern was related to changes in respiration (Bossio et al., 1998), which may be linked to total lignocellulase activity. However, the correlation between total

enzyme activity and community composition may be due to concurrent changes in the microbial biomass or labile C. Therefore, we found it valuable to normalize enzyme activities to microbial biomass. We used the specific activity of the enzymes as a measure of physiological capacity, assuming that the enzymes assayed were representative of the activities of the currently or recently viable microbial community.

The specific activity of all enzymes (except sulphatase and β -glucosidase) was more closely related to community composition than total enzyme activity. The specific activity of cellobiohydrolase, phenol oxidase, peroxidase, and phosphatase specific activities were correlated with a larger number of fatty acids than were β -glucosidase, β -xylosidase, or sulphatase. This result may in part be due to the function of the enzymes. Phenol oxidase and peroxidase are primarily responsible for lignin degradation while cellobiohydrolase is responsible for cellulose degradation. Cellobiohydrolase differs from β -glucosidase and β -xylosidase primarily in that cellobiohydrolase releases dimers from the cellulose strand rather than monomers. Thus, the enzymes that degrade larger macromolecular substrates may be more closely related to community composition than enzymes that degrade simpler substrates. These results suggest that phenol oxidase, peroxidase, and cellobiohydrolase specific activities are more strongly related to shifts in community composition than β -glucosidase and β -xylosidase. It is also interesting that the specific activity of phosphatase was also correlated with a large number of individual fatty acids and sulphatase was not. The majority of microbes produce phosphatase, but it is possible that only a portion of the microbial community may produce phosphatase at any one time (Hysek and Sarapatka, 1998). Because sulphatase was highly correlated with microbial biomass but the specific activity of sulphatase was not correlated with community composition, sulphatase is most likely produced by a very broad range of microbial populations.

The specific activity of β -glucosidase and β -xylosidase were correlated with monounsaturated fatty acids, typical of Gram-negative bacteria. These enzymes release simple sugars, and Gram-negative bacteria are often fast growers, which compete for simple substrates (Atlas and Bartha, 1993). In contrast, cellobiohydrolase, phenol oxidase, and peroxidase were correlated with fatty acids typical of Gram-positive and Gram-negative bacteria, fungi and the actinomycetes. Gram-positive bacteria have sometimes been considered to be stress tolerators that grow slowly and tend to be able to metabolize complex carbon substrates more readily than Gram-negative bacteria (Paul and Clark, 1989; Halverson et al., 2000). Fungi and actinomycetes are commonly considered to be producers of oxidative enzymes. Thus, subsets of the microbial community identified by PLFA biomarker analysis can be related to function.

Differences in community composition as a result of land use change may alter the amount of enzyme produced per

unit of biomass. This has interesting implications for C cycling research. It is common for process-oriented carbon cycling models to assume that microbial activities are constant for a given amount of biomass (Cole et al., 1993; Paustian et al., 1992). Our results suggest that this is not necessarily the case. Compositional changes in microbial communities are accompanied by changes in their capabilities to degrade macromolecular carbon compounds. An explicit index of specific functional groups of soils organisms, such as the lignocellulase degrading community, may be useful in models to improve prediction of the effects of environmental change (Smith et al., 1998). Better understanding of the link between enzyme activity and community composition may help provide microbial community parameters that can be related to potential rates of degradation of macromolecular carbon compounds.

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References

- Andr n, O., Brussaard, L., Clarholm, M., 1999. Soil organism influence on ecosystem-level process — bypassing the ecological hierarchy? *Applied Soil Ecology* 11, 177–188.
- Atlas, R.M., Bartha, R., 1993. *Microbial Ecology: Fundamentals and Applications*. Benjamin Cummings, Redwood City, CA (563pp).
- Balser, T.C., Kinzig, A., Firestone, M.K., 2000. Linking soil microbial communities and ecosystem functioning. In: Kinzig, A., Parala, S., Tilman, D. (Eds.). *Biodiversity and Ecosystem Functioning*. Princeton University Press, Princeton. (in press).
- Berg, B., McClaugherty, C., Virzo De Santo, A., Johansson, M.B., Ekbohm, G., 1995. Decomposition of litter and soil organic matter — can we distinguish a mechanism for soil organic matter buildup? *Scandinavian Journal of Forest Research* 10, 108–119.
- Bossio, D.A., Scow, K.M., 1995. Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. *Applied and Environmental Microbiology* 61, 4043–4050.
- Bossio, D.A., Scow, K.M., Gunapala, N., Graham, K.J., 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology* 36, 1–12.
- Buyer, J.S., Drinkwater, L.E., 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. *Journal of Microbiological Methods* 30, 3–11.
- Cole, C.V., Paustian, K., Elliott, E.T., Metherell, A.K., Ojima, D.S., Parton, W.J., 1993. Analysis of agroecosystem carbon pools. *Water Air and Soil Pollution* 70, 357–371.
- Degens, B.P., 1998. Decreases in microbial functional diversity do not result in corresponding changes in decomposition under different moisture conditions. *Soil Biology & Biochemistry* 30, 1989–2000.
- Degens, B.P., 1999. Catabolic response profiles differ between microorganisms grown in soils. *Soil Biology & Biochemistry* 31, 475–477.
- Dick, W.A., Tabatabai, M.A., 1992. Significance and potential uses of soil enzymes. In: Metting, F.J.B. (Ed.). *Soil Microbial Ecology: Applications in Agriculture and Environmental Management*. Marcel Dekker, New York, pp. 95–125.
- Dick, R.P., 1994. Soil enzyme activities as indicators of soil quality. In: Doran, J.W. (Ed.). *Defining Soil Quality for a Sustainable Environment*. Soil Science Society of America, Madison, WI, pp. 107–124.
- Dick, R.P., Sandor, J.A., Eash, N.S., 1994b. Soil enzyme activities after 1500 years of terrace agriculture in the Colca Valley, Peru. *Agriculture Ecosystems & Environment* 50, 123–131.
- Falcon, M.A., Rodriguez, A., Carnicero, A., Regalado, V., Perestelo, F., Milstein, O., Fuente, G.D.L., 1995. Isolation of microorganisms with lignin transformation potential from soil of Tenerife island. *Soil Biology & Biochemistry* 27, 121–126.
- Federle, T.W., Livingston, R.J., Wolfe, L.E., White, D.C., 1986. A quantitative comparison of microbial community structure of estuarine sediments from microcosms and the field. *Canadian Journal of Microbiology* 32, 319–325.
- Fernandez, A., Huang, S., Seston, S., Xing, J., Hickey, R., Criddle, C., Tiedje, J., 1999. How stable is stable? Function versus community composition. *Applied and Environmental Microbiology* 65, 3697–3704.
- Garland, J.L., 1996. Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. *Soil Biology & Biochemistry* 28, 213–221.
- Halverson, L.J., Jones, T.M., Firestone, M.K., 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. *Soil Science Society of America Journal* 64 (in press).
- Hammel, K.E., 1997. Fungal degradation of lignin. In: Cadish, G., Giller, K.E. (Eds.). *Driven by Nature: Plant Litter Quality and Decomposition*. CAB International, Wallingford, UK, pp. 33–45.
- Holden, P.A., Firestone, M.K., 1997. Soil microorganisms and soil cleanup: how can we improve our understanding? *Journal of Environmental Quality* 26, 32–40.
- Hu, S., van Bruggen, A.H.C., 1997. Microbial dynamics associated with multiphasic decomposition of ¹⁴C-labeled cellulose in soil. *Microbial Ecology* 33, 134–143.
- Hysek, J., Sarapatka, B., 1998. Relationship between phosphatase active bacteria and phosphatase activities in forest soils. *Biology and Fertility of Soils* 26, 112–115.
- Kennedy, A.C., Smith, K.L., 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant and Soil* 170, 78–86.
- Lang, E., Eller, G., Zadrazil, F., 1997. Lignocellulose decomposition and production of ligninolytic enzymes during interaction of white rot fungi with soil microorganisms. *Microbial Ecology* 34, 1–10.
- Mehlich, A., 1984. Mehlich 3 soil test extractant: a modification of Mehlich 2. *Communications in Soil Science and Plant Analysis* 15, 1409–1416.
- Paul, E.A., Clark, F.E., 1989. *Soil microbiology and biochemistry*. Academic, San Diego, CA (273pp).
- Paustian, K., Parton, W.J., Persson, J., 1992. Modeling soil organic matter in organic-amended and nitrogen fertilized long term plots. *Soil Science Society of America Journal* 56, 476–488.
- Schimel, J., 1995. Ecosystem consequences of microbial diversity and community structure. In: Chapin, F.S., Koerner, C. (Eds.). *Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences*. Springer, Berlin, pp. 239–254.
- Sinsabaugh, R.L., Antibus, R.K., Linkins, A.E., McClaugherty, C.A., Rayburn, L., Repert, D., Weiland, T., 1993. Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. *Ecology (Tempe)* 74, 1586–1593.
- Smalla, K., Wachtendorf, U., Heuer, H., Liu, W.-T., Forney, L., 1998. Analysis of Biolog GN substrate utilization patterns by microbial communities. *Applied and Environmental Microbiology* 64, 1220–1225.
- Smith, P., Andren, O., Brussaard, L., Dangerfield, M., Ekschmitt, K., Lavelle, P., Tate, K., 1998. Soil biota and global change at the ecosystem level: describing soil biota in mathematical models. *Global Change Biology* 4, 773–784.

- Tate, R.L., 1995. *Soil Microbiology*. Wiley, New York (398pp).
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry* 19, 703–707.
- Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology — quantitative approaches to the study of microbial communities. *Bioscience* 39, 535–541.
- White, D.C., Ringelberg, D.B., 1998. Signature lipid biomarker analysis. In: Burlage, R.S., Atlas, R., Stahl, D., Geesey, G., Saylor, G. (Eds.). *Techniques in Microbial Ecology*. Oxford, New York, pp. 255–272.
- Wilkinson, S.G., 1988. Gram-Negative Bacteria. In: Ratledge, C., Wilkinson, S.G. (Eds.). *Microbial Lipids*. Academic, London, pp. 299–488.
- Zak, J.C., Willig, M.R., Moorhead, D.L., Wildman, H.G., 1994. Functional diversity of microbial communities: a quantitative approach. *Soil Biology & Biochemistry* 26, 1101–1108.
- Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald, N.W., Pregitzer, K.S., White, D.C., 1997. Compositional and functional shifts in microbial communities due to soil warming. *Soil Science Society of America Journal* 61, 475–481.