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VEGETATION REMOVAL IN TWO SOILS OF THE HUMID TROPICS: EFFECT ON MICROBIAL BIOMASS

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Summary—Conversion of forests of the humid tropics into pastures and agricultural fields is expected to produce, in the long-term, a decline in soil organic matter content and soil fertility. Changes in microbial biomass (Biomass C, Bc) following vegetation removal can provide an early indication for slower, less easily detectable SOM changes. Microbial biomass can also provide an index of soil fertility because it represents an important labile pool of soil nutrients and plays an active role in preventing nutrient loss. There are few published measurements of Bc in the humid tropics and fewer of Bc changes due to shifts in vegetation cover. We measured Bc in two humid tropical soils (an oxic Humitropept at 4.5% C and pH of 5.0 and a fluventic Dystrandept at 2.6% C and pH of 6.4) subjected, for 3 yr, to extreme treatments: soil maintained bare, annual harvest of re-growth, and native 20 yr old secondary vegetation. Both soils showed a similar pattern in total SOM and Bc decline following vegetation removal: after 3 yr, total C and N were reduced by 20%. Response of Bc was more pronounced. In the bare soil, most of the decline in Bc occurred within the first 6 months (to 50% of initial values) and after 15 months, Bc appeared to have stabilized at *ca* 35% of its initial value. Response of Bc to the annual harvest treatment was more moderate than to the bare soil treatment. Determining the precise size of microbial biomass is difficult because of variability in time and differences between techniques, but Bc values determined by two techniques and on numerous dates were high: in control plots around 2000 (oxic Humitropept) and 1300 (fluventic Dystrandept) μg C g^{-1} soil (or 250 and 187 g m^{-2} after correction for differences in bulk density), which suggests that Bc in humid tropical soils can be high. Microbial C represented *ca* 4% of the total C in the control and declined to *ca* 1.5% of the total C in the bare soil. Eucaryote:procaryote ratios were close to 1 for all treatments except in the Fluventic Dystrandept control, which supported a woody vegetation and had a ratio of 3.3. This study demonstrates the dynamic nature of microbial biomass following tropical forest clearing and its potential importance for affecting nutrient loss.

INTRODUCTION

Large changes in vegetation cover are taking place in the humid tropics as forests are converted to pastures and agricultural fields. An expected result from the removal of tree biomass and the change in land use is, in the long-term, a decline in soil organic matter (SOM). Soil organic matter is of crucial importance for maintaining the fertility of highly weathered tropical soils and for insuring the sustainability of agricultural systems developed on these soils. Indeed, in soils dominated by kaolinitic and other clays with variable charge, SOM is responsible not only for supplying nutrients but also for retaining them because it provides most of the soil cation exchange capacity (Uehara and Gillman, 1981).

Current models of SOM dynamics, e.g. CENTURY, divide SOM into three fractions with different turnover times: the active (0.14 yr), slow (5 yr) and passive (150 yr) fractions (Parton *et al.*, 1989). Because of differences in turnover times, the response of total SOM to a change in vegetation cover and soil management could escape detection for decades whereas the response of the active fraction (and a decrease in soil fertility) can occur much more rapidly (Powlson *et al.*, 1987). The active fraction with short turnover time appears to contain primarily the living soil microbial biomass and microbial products (Parton *et al.*, 1989).

Information on changes in microbial biomass following vegetation removal is valuable not only because it provides an indication on slower, less easily detectable, SOM changes but also because microbial biomass contributes to soil fertility: it represents an important labile pool of soil nutrients and may play in many systems an active role (through immobilization) in preventing nutrient leaching (e.g. Vitousek and Matson, 1984).

In spite of the recognized role of microbial biomass in SOM and nutrient dynamics in humid tropical soils, there are few published measurements of soil microbial biomass in the humid tropics, and fewer still of soil microbial biomass changes due to shifts of vegetation cover (Ayanaba *et al.*, 1976; Dash *et al.*, 1985; Bonde, 1991; Luizão *et al.*, 1992).

We have investigated microbial biomass and microbial biomass changes in two soils of the humid tropics subjected, for 3 yr, to extreme vegetation

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Table 1. Soil properties 153 weeks (14 March 1990) after the onset of the vegetation removal experiment

	Oxic Humitropept			Fluventic Dystrandept		
	Control	Annual harvest	Bare	Control	Annual harvest	Bare
C (%)	4.53 ± 0.56	4.06 ± 0.16	3.87 ± 0.78	2.62 ± 0.09	2.38 ± 0.12	1.78 ± 0.12
Percent of control C		89.6	85.4		90.8	67.9
N (%)	0.28 ± 0.03	0.23 ± 0.04	0.19 ± 0.04	0.20 ± 0.02	0.19 ± 0.03	0.16 ± 0.02
Percent of control N		82.1	67.8		95.0	80.0
C:N ratio	16.0 ± 2.2	17.6 ± 2.3	20.1 ± 2.5	12.9 ± 0.8	12.6 ± 0.7	11.3 ± 1.0
pH	4.98 ± 0.08	4.70 ± 0.24	4.53 ± 0.17	6.45 ± 0.04	6.35 ± 0.08	6.36 ± 0.1

removal treatments. Microbial biomass in plots with bare soil or with vegetation harvested annually were compared to microbial biomass in 20 yr old secondary forest stands.

Further, we tested the hypothesis that the difference in organic inputs between the treatments induced a shift in the microbial populations: from fungi-dominated in the secondary forest to bacteria-dominated in the bare soil. Such a shift would be expected to decrease the C:N ratio of the active SOM pool and modify its mineralization pattern.

MATERIALS AND METHODS

Study site and vegetation removal treatments

The study site was located in the lowlands of Costa Rica, Sarapiqui province at the Organization for Tropical Studies' La Selva Biological Station (4.3 m mean annual rainfall, minimum and maximum annual temperature of 20.9 and 30.9°C, respectively). Four blocks of three plots (13 × 13 m) were established on each of two contrasting river terraces. The lower terrace is underlain by a fluventic Dystrandept soil and the upper terrace, older and less fertile, by an oxic Humitropept. Selected soil properties are given in Table 1. On both terraces stood a 20 yr old secondary vegetation of 2 m high ferns and grasses, thickets of woody shrubs, and scattered stands of trees; trees and shrubby vegetation were, however, more abundant on the lower terrace.

In April 1987, vegetation was removed from two plots in each block. In one of these plots further regrowth was prevented by hand-weeding biweekly (bare fallow plot) whereas in the other, vegetation was harvested and removed from the site annually (annual harvest plot). In the third plot of each block, secondary vegetation was left untouched (control plot).

Soil sampling

Soil samples for microbial biomass measurements were collected from the 24 plots to 0.15 m depth regularly from April 1987 to May 1989, then on 15 March 1990 (at the end of the dry season and a few days after the third annual vegetation removal from the vegetated plots) and 6 months later, during the wet season, on 30 October 1990. For the routine sampling and in March 1990, one composite soil sample was taken per plot, in October 1990, three. The samples were homogenized in plastic bags by kneading and roots were removed with forceps. Extractions and incubations were performed on-site, the same day as soil collection. Soil water content at the time of sampling, for all treatments and sample dates was between 35 and 40% for the oxic Humitropept and 30 and 35% for the fluventic Dystrandept.

Microbial biomass measurements

Fumigation-direct extraction (FE). Soil samples (15 g fresh wt) were incubated for 10 days in sealed glass jars (0.1 l.) with 1 ml chloroform. Chloroform was removed by ventilation and the samples were ex-

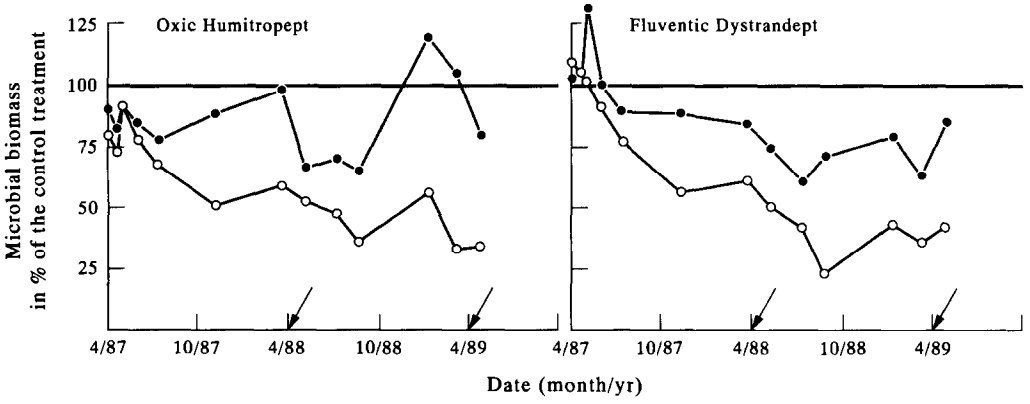

Fig. 1. Microbial biomass, expressed as percentage of the control treatment values, for the first 2 yr of the experiment: (●) annual harvest; (○) bare soil treatment. The arrows indicate the dates of the annual harvest. See Table 2 for absolute values for microbial biomass.

Table 2. Biomass C (μg C g^{-1}l soil dry wt) determined by fumigation–extaction and fumigation–incubation. Values are means of four replicate blocks per treatment ± standard errors

	Oxic Humitropept			Fluventic Dystrandept		
	Control	Annual harvest	Bare	Control	Annual harvest	Bare
Fumigation–extraction						
March 1990	1936 ± 416[a]	1113 ± 152[b]	596 ± 84[c]	762 ± 73[a]	577 ± 52[b]	295 ± 36[c]
Percent of control		57.4	30.7		75.7	38.7
Percent of total C	4.27	2.74	1.54	2.90*	2.42	1.65
October 1990	2386 ± 206[a]	1347 ± 444[b]	1115 ± 463[b]	1290 ± 256[a]	757 ± 221[b]	110 ± 71[c]
Percent of control		56.4	46.7		58.6	8.5
Fumigation–incubation						
March 1990	2073 ± 1123[a]	1616 ± 87[a]	1146 ± 144[a]	946 ± 190[a]	1253 ± 204[b]	659 ± 119[c]
Percent of control		77.9	55.2		132.4	69.6
October 1990	2116 ± 507[a]	1290 ± 332[b]	1217 ± 382[b]	1196 ± 397[a]	1015 ± 59[ab]	776 ± 120[b]
Percent of control		60.9	57.5		84.8	64.8

*4.90 if the October value (1290) is used instead of the March value (762).
a, b, c: Fisher PLSD significant at 95% within soil and date.

tracted overnight with 100 ml of 1 M KCl. Ninhydrin-reactive N in the filtered extracts was measured by reacting aliquots of extracts with a ninhydrin reagent and reading adsorbance at 570 nm. Biomass C (Bc) was estimated from the amount of ninhydrin-reactive N released by fumigation: Bc = 21 × release of ninhydrin-reactive N (Amato and Ladd, 1988).

Fumigation-incubation (FI). One set of samples (15 g fresh wt) was fumigated for 6 h in sealed glass jars (0.1 l.) with 1 ml chloroform (fumigated soil). Chloroform was removed and the samples were transferred to 0.4 l. sealed canning jars for 10 days. A second set of samples (15 g in open canning jars) was incubated for 10 days before the jars were sealed for 10 days (control). At the end of the incubation period, gas aliquots were taken from the jar headspaces and the soil was extracted with 100 ml of 1 M KCl. Carbon dioxide in the headspace was measured by infrared gas analysis and NH_4^+-N and NO_3^--N concentrations in the filtered extracts were determined with an autoanalyzer. Biomass C was estimated by the flux of CO_2-C from fumigated samples compared to controls: Bc = {(CO_2-C evolved by fumigated soil in 0–10 days) – (CO_2-C evolved by control in 10–20 days)}/K_c; using K_c = 0.45 (Jenkinson *et al.*, 1976). Mineralized N = {(NH_4^+-N + NO_3^--N mineralized by fumigated soil in 0–10 days) – (NH_4^+-N + NO_3^--N mineralized by control in 10–20 days)}.

Biomass C estimates in μg C g^{-1} soil dry wt were converted in g C m^{-2} by multiplying estimates by 0.150 (fluventic Dystrandept) and 0.105 (oxic Humitropept) to account for the difference in bulk density between the two soils (1.0 and 0.7 g cm^{-3}, respectively).

Eucaryote:procaryote ratios

Composite soil samples were collected on 22 November 1990, from each plot and brought back (in a cooler box with ice), moist, to the Kellogg Biological station (U.S.A.) for analyses. Eucaryote:procaryote ratios were estimated by the selective respiratory inhibition technique as described by West (1986). Triple-replicated soil aliquots (1.2 g fresh wt) were placed in 38 ml glass vials sealed with rubber septas and to which were added 2 ml of a glucose solution (100 mg glucose g^{-1} soil dry wt) containing either no antibiotic (control), cycloheximin (50 mg

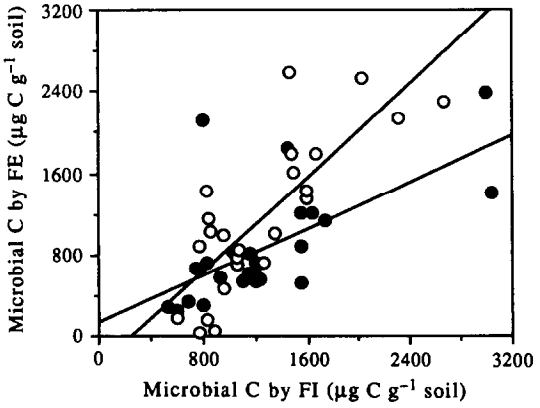

Fig. 2. Correlation between microbial biomass determined by fumigation–incubation (FI) and fumigation–direct extraction (FE) in samples from March 1990 [(●), r^2 = 0.40] and October 1990 [(○), r^2 = 0.64].

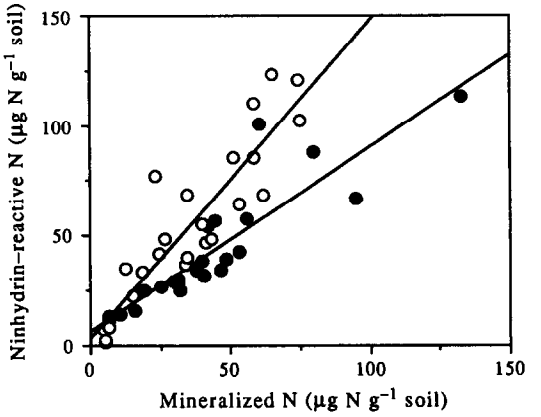

Fig. 3. Correlation between N mineralized during incubation after fumigation (FI) and ninhydrin-reactive N released by fumigation (FE) in samples from March 1990 [(●), r^2 = 0.80] and October 1990 [(○), r^2 = 0.81].

Table 3. Ratio of C–CO_2 flush:N mineralized during incubation after fumigation

	Oxic Humitropept			Fluventic Dystrandept		
	Control	Annual harvest	Bare	Control	Annual harvest	Bare
March 1990	9.9 ± 3.8^{b}	14.5 ± 3.9^{ab}	21.2 ± 8.4^{a}	9.7 ± 2.4^{c}	20.6 ± 4.2^{b}	32.4 ± 8.7^{a}
October 1990	14.2 ± 4.5^{a}	12.5 ± 1.8^{a}	13.0 ± 2.5^{a}	16.6 ± 8.6^{b}	19.7 ± 8.8^{ab}	67.3 ± 18.8^{a}

a, b, c: Fisher PLSD significant at 95% within soil and date.

g^{-1} soil), or streptomycin (200 mg g^{-1} soil). The vials were shaken and incubated for 5 h at 25°C. Gas aliquots were taken from the headspace and CO_2 determined by infrared gas analysis.

Eucaryote:procaryote ratios were calculated as [CO_2 in control − CO_2 in streptomycin treatment]/[CO_2 in control − CO_2 in cycloheximin treatment].

RESULTS AND DISCUSSION

Effect of vegetation removal on total SOM and microbial biomass (Bc)

Although the oxic Humitropept had initially nearly twice the C content of the fluventic Dystrandept (4.5 vs 2.6%), both soils showed a relatively similar pattern in total SOM decline, with a loss of *ca* 20% of the total C and N after 3 yr of the vegetation removal treatment (Table 1).

Response of Bc to the treatments was more pronounced but also followed a similar pattern in the two soils (Fig. 1). In the bare soil, most of the decline in Bc occurred within the first 6 months (to 50% of the initial value). Further decline was slower and after 15 months, Bc had stabilized at around 35% of its initial value (Fig. 1).

Response of Bc to the annual harvest treatment was more moderate than to the bare soil treatment (Fig. 1). Only in the oxic Humitropet was it possible to see some indication of increase of Bc during the growing season and decline after harvest.

Changes in Bc in our treatments were relatively rapid. Data available in the literature for comparison are few and comparison difficult. Ayanaba *et al.* (1976) reported the loss of Bc after conversion of a bush regrowth to an agricultural field in an Alfisol of S.W. Nigeria (pH of 6.8, 1.1% total C): after 2 yr of cultivation, they observed a decrease of 30% of Bc in the cultivated treatment with no crop and of 25 (no NPK added) or 15% (NPK added) in the maize treatment (no residue added); Bc in the bush fallow was 270 μg C g^{-1} soil. Bonde (1991) and Luizão *et al.* (1992) reported the change in Bc after conversion of a virgin rainforest to pasture in an Amazonian Oxisol (pH of 3.7, 4.8% total C): no change after 1 yr (0–5 cm depth, Luizão *et al.*, 1992), 16% (0–3 cm) and 30% (3–10 cm) after 2 yr, and 42% (0–3 cm) and 58% (3–10 cm) after 8 yr (Bonde, 1991); Bc of the virgin forest was 1463 ± 121 (0–3 cm) and 1046 ± 110 (3–10 cm) μg C g^{-1} soil.

Size of Bc estimated by fumigation–direct extraction (FE) and fumigation–incubation (FI)

Methodologies to quantify Bc are still subject to controversies concerning the handling of the samples (e.g. pre-incubation, re-inoculation of the fumigated soils) and the factors used to convert C and N flushes to Bc (e.g. $K_c = 0.45$ or other). From April 1987 to May 1989, Bc was determined only by the fumigation–extraction method. In March and October 1990, we estimated Bc by fumigation–extraction (FE) and fumigation–incubation (FI), using the procedure and calculation factors outlined above.

In contrast to Vance *et al.* (1987), who had failed to observe a significant C flush from acid temperate forest soils, we observed vigorous respiration in the fumigated samples of our acid soil (oxic Humitropept, pH 4.5) without re-inoculation of the soil prior to incubation. Similar observations were made by Luizão *et al.* (1992) and Bonde (1991). The notion that the FI method is ineffective for Bc measurement in acid forest soils should therefore be re-examined, at least for tropical soils.

Microbial biomass in the oxic Humitropept was higher than in the fluventic Dystrandept for all treatments (Table 2; $P < 0.001$), which corresponds to our expectation since the oxic Humitropept has a higher total C content and microbial biomass correlates generally well with total C (Theng *et al.*, 1989).

Both FI and FE techniques gave values of Bc which are at the upper limit of values reported for soils of the temperate zone. Microbial biomass values in our control treatments were close to 2000 (oxic Humitropept) and 1300 (fluventic Dystrandept) μg C g^{-1} soil (Table 2), or 250 and 187 g m^{-2} after correction for differences in bulk density. The range of values for temperate forest soils is 720–1900 μg C g^{-1} (Vance *et al.*, 1987) and generally lower for cultivated soils. The only published values (to our knowledge) of Bc measured in a similar environment (forests and pastures on a Brazilian typical

Table 4. Eucaryote:procaryote ratios

Oxic Humitropept			Fluventic Dystrandept		
Control	Annual harvest	Bare	Control	Annual harvest	Bare
1.20 ± 0.09	1.01 ± 0.24	1.01 ± 0.14	3.31 ± 0.25	1.30 ± 0.91	1.04 ± 0.82

Acrorthox) are also high, around 1600 μg g^{-1} soil (Bonde, 1991; Luizão *et al.*, 1992).

Our data and the data from the Brazilian site (Bonde, 1991; Luizão *et al.*, 1992) counter the conventional wisdom that Bc is low in tropical soils (Theng *et al.*, 1989). Clearly more data are needed before we can make a general statement concerning Bc in the soils of the humid tropics.

Microbial biomass in the bare soil, although significantly lower than in the control, remains high: 300–600 μg g^{-1} soil (Table 2). Since the bare soil received little or no organic input (only rootlets of seedlings that developed on the plots between weeding events and the occasional leaf from adjacent plots), these Bc values indicate that after 3 yr of extreme treatment, there remains a sufficient amount of non-recalcitrant SOM (e.g. from decaying roots) or, alternately, that the remaining microbial population can utilize the more recalcitrant C sources.

Using values of Bc and total soil C determined in March 1990, we estimated that Bc represented *ca* 4% of the total C in the control treatments and declined to *ca* 1.5% of the total C in the bare soil (Table 2). Again, our data are similar to patterns found by Luizão *et al.* (1992) in Amazonian pastures and forest (where Bc represented 3.5–5.3% of total C), and falls in the higher limit of the range reported in the literature for temperate systems: 0.27–5.0 for cultivated soils (Anderson and Domsch, 1986) and 1.8–2.9 for forest soils (Vance *et al.*, 1987).

Although both FI and FE techniques agreed on a significant decline of Bc with treatment intensity (Bc in control > annual harvest > bare) (Table 2), Bc estimated by FE and FI correlated relatively poorly ($r = 0.8$; Fig. 2). In contrast, ninhydrin-reactive N liberated by fumigation (FE) and N mineralized during incubation of the fumigated soil (FI) showed a much better correlation ($r = 0.9$; Fig. 3).

This suggests that the N pool measured by the two techniques is the same but that the C respired per unit N mineralized is not constant. Indeed, the ratio of $C:CO_2$ flush over N mineralized during incubation after fumigation generally increases in the order control < annual harvest < bare treatment (Table 3). Bacteria have an expected C-to-N ratio in the range of 3–5 and fungi in the range of 15–4.5 (Paul and Clark, 1989), therefore all our values are high if they are indicative of the C:N ratio of the microbial biomass, especially considering that eucaryote:procaryote ratios for most samples were close to 1 (see later and Table 4). A tentative explanation is that, in the bare soil, the microbial populations recolonizing the soil after fumigation not only mineralize the microbial biomass killed by fumigation but also very recalcitrant (high C:N) soil organic matter.

Although the trends in Bc in response to the treatments are clear, the accurate size of Bc is not easily determined. Differences in Bc values between techniques and between sampling dates can be substantial (Table 2). Microbial biomass is a dynamic pool and its size is probably significantly affected by climatic events such as rainfall or drought period. A one-time measurement of Bc using a single technique would therefore provide a poorly reliable estimate of Bc.

Effect of vegetation removal on eucaryote:procaryote ratio

Eucaryote:procaryote ratios were close to 1 for all treatments except for the control treatment of the fluventic Dystrandept (Table 4). In the oxic Humitropept, there appears therefore to be no change in fungi to bacteria balance with vegetation removal. This is probably due to the fact that secondary vegetation on this soil was predominantly tall grasses and ferns.

The secondary vegetation developed on the fluventic Dystrandept control was predominantly woody and the microbial population was fungi-dominated (eucaryote:procaryote ratio of 3.3). Vegetation removal induced a decline of the fungal contribution in both the annual harvest and bare soil treatments.

Our study demonstrated the dynamic nature of Bc following tropical forest clearing and its potential importance for affecting soil fertility since Bc represents an important labile pool of soil nutrients and may play an active role in preventing nutrient losses. Decline of Bc may provide an early indication for slower, less-easily detectable SOM changes.

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