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SOIL MICROBIOLOGY

Comparative Resistance and Resilience of Soil Microbial Communities and Enzyme Activities in Adjacent Native Forest and Agricultural Soils

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Abstract Degradation of soil properties following deforestation and long-term soil cultivation may lead to decreases in soil microbial diversity and functional stability. In this study, we investigated the differences in the stability (resistance and resilience) of microbial community composition and enzyme activities in adjacent soils under either native tropical forest (FST) or in agricultural cropping use for 14 years (AGR). Mineral soil samples (0 to 5 cm) from both areas were incubated at 40°C, 50°C, 60°C, or 70°C for 15 min in order to successively reduce the microbial biomass. Three and 30 days after the heat shocks, fluorescein diacetate (FDA) hydrolysis, cellulase and laccase activities, and phospholipid-derived fatty acids-based microbial community composition were measured. Microbial biomass was reduced up to 25% in both soils 3 days after the heat shocks. The higher initial values of microbial biomass, enzyme activity, total and particulate soil organic carbon, and aggregate stability in the FST soil coincided with higher enzymatic stability after heat shocks. FDA hydrolysis activity was less affected (more resistance) and cellulase and laccase activities recovered more rapidly (more resilience) in the FST soil relative to the AGR counterpart. In the AGR soil, laccase activity did not show resilience to any heat shock level up to 30 days after the disturbance. Within each soil type, the microbial community composition did not differ between heat shock and control samples at day 3. However, at day 30, FST soil samples treated at 60°C and 70°C contained a microbial community significantly different from the control and with lower biomass regardless of high enzyme resilience. Results of this study show that deforestation followed by long-term cultivation changed microbial community composition and had differential effects on microbial functional stability. Both soils displayed similar resilience to FDA hydrolysis, a composite measure of a broad range of hydrolases, supporting the concept of high functional redundancy in soil microbial communities. In contrast, the resilience of the substrate-specific activities of laccase and cellulase were lower in AGR soils, indicating a less diverse community of microorganisms capable of producing these enzymes and confirming that specific microbial functions are more sensitive measurements for evaluating change in the ecological stability of soils.

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Introduction

The removal of natural vegetation followed by cultivation can cause severe changes to physical, chemical, and biological soil properties. These changes are often associated with reduction of soil organic matter, deterioration of soil structure, and decreases in microbial biomass and activity [3, 16, 27, 40, 46, 47, 49]. Many studies have also shown that deforestation and soil cultivation alter soil microbial community structure [2, 3, 41, 49] and may lead to reduction in microbial biodiversity [8, 14].

It is uncertain how deterioration of soil properties and changes in microbial communities affect the functional stability of soils. Stability comprises both resistance, i.e., the ability to withstand a perturbation or stress, and resilience, i.e., the ability to recover to preperturbation levels [22]. It has been hypothesized that soil functional stability is primarily generated by the inherent functional redundancy present in the microbial diversity [13, 21, 22, 48]. This hypothesis is referred to as the "insurance hypothesis of biodiversity" [32, 36] and was originally proposed for macroorganisms [17, 33].

Few studies have tried to validate the insurance hypothesis in soil environments [19, 22, 23]. Overall, the data indicate that the effect of stress or disturbance on the functional stability of soils depends on the level of specificity of the function. For example, broad-scale functions such as decomposition of organic matter are generally not affected by microbial diversity, whereas more specialized niche functions such as nitrification, denitrification, methane oxidation, and mineralization of xenobiotics decrease as biodiversity decreases [19, 22].

Studies investigating the effects of soil cultivation on functional stability are scarce. Degens et al. [13] showed that an arable soil with low catabolic diversity and organic C was less resistant to stress (low pH, high salinity, heavy metal contamination) or disturbance (wet–dry or freeze–thaw cycles) than a similar soil type under pasture where catabolic diversity and organic C were higher. In another study, Griffiths et al. [23] found greater functional stability (short-term decomposition of plant residues) to copper and heat stresses in a grassland soil and an organically managed agricultural soil than in an intensively managed soil.

To date, we are unaware of any studies that compared the functional stability of a forest soil that was brought into long-term cultivation with its uncultivated control. In this work, the soil functional stability of a natural forest and an adjacent agricultural site was evaluated after sequential reduction of microbial biomass by heat shock disturbances (40°C to 70°C). It was expected that decreasing the overall biomass by these treatments would also cause a decrease in functional redundancy, especially for those functions associated with more specialized microbial groups. Soil functional stability was measured by evaluating a "broad-scale" soil enzyme (hydrolysis of fluorescein diacetate [FDA]) and two "narrow-niche" soil enzymes (cellulase, EC 3.2.1.4, and laccase, EC 1.10.3.2) in parallel with changes in soil microbial community composition using phospholipid-derived fatty acids (PLFA) profiling. We hypothesized that the rate of recovery of these enzyme activities after the heat shocks would differ between the two soils and that the difference between them would be related to the composition of the microbial communities.

Methods

Sampling Sites

The study soils represent a fine-loamy, kaolinitic, isohyperthermic Typic Fragiudult collected from a Brazilian Atlantic Forest fragment (FST) and from an adjacent agricultural field (AGR) that were less than 200 m apart. Both sites are located in the Umbaúba Experimental Station of the Embrapa Coastal Tablelands in Sergipe State, Northeastern Brazil (11°16′ S, 37°26′ W, 105 m altitude; 1,350 mm of annual rainfall; 24.3°C mean daily temperature). The FST is about 700×300 m in dimension and has been isolated from anthropogenic intervention for more than 50 years. The site is occupied mainly by midsuccessional tree species and shrubs. During the 14 years preceding the soil sampling, AGR was in continuous cultivation of single-cropped corn (*Zea mays*) under conventional tillage (one moldboard plowing and two diskings every year) from 1991 through 2000 and cultivated with intercropped corn and pigeon pea (*Cajanus cajan*) under zero-tillage from 2001 through 2004.

Soil Sampling and Characterization

At each site, 40 random soil cores were collected from 0 to 5 cm depth and pooled to form one composite sample. Samples were passed through a 4-mm sieve, air-dried, and stored at 4°C. Soil pH was measured in a slurry of soil and water (1:2.5). Total organic carbon (TOC) was quantified by ignition in a LECO WR-12 C autoanalyzer (LECO, St. Joseph, MO, USA). A 25-g soil subsample was used to obtain particulate SOM by a procedure adapted from Cambardella and Elliot [10]. The soil was dispersed in 100 mL of sodium metahexaphosphate (5 g L^{-1}) adjusted to pH 9 to improve dispersion of colloids in this low-pH soil. Aggregates were disrupted by agitation of soil suspensions in a rotatory shaker (250 rpm) for 8 h. The slurry was passed through a 0.106-mm sieve; the portion retained was washed with excess distilled water and dried at 60°C for 24 h. The TOC in the dried fraction was determined as described above. Results are expressed as the percentage of C as particulate organic matter in the whole soil.

Extractable P (Mehlich-1) was determined by a published procedure [5]. Water-stable aggregates (WSA) were determined according to Nimmo and Perkins [38] using a single-sieve wet-sieving apparatus with a 0.106-mm sieve and NaOH (2 g L^{-1}) as dispersing agent. The weighed mean diameter of soil aggregates [29] was determined after sorting the aggregates by dry-sieving [35]. Soil texture was determined by the Bouyoucos densimeter method with hexametaphosphate as the dispersing agent [4].

Heat-Induced Microbial Biomass Reduction

Soil water content was adjusted to 60% of the water-holding capacity (WHC), and each soil type was divided into 40 portions, each equivalent to 11 g (wet weight), and placed into 25 mL flasks. This provided 80 experimental units for four replicates of five levels of heat shock and two dates of evaluation for each soil. Samples were preincubated for 7 days at 25°C in a dark room before disturbance application. After the preincubation period, samples of FST and AGR soils were exposed to 40°C, 50°C, 60°C, or 70°C (plus a control, kept at 25°C). The higher temperature levels used are similar to those used to pasteurize soil and were chosen to assure some reduction of microbial biomass. For each soil type, eight-flask subsets were partially immersed into a heated water bath. The total period of immersion equaled the sum of the time for the soil temperature to reach 95% of the nominal temperature of the water bath (e.g., for the water bath set at 40°C, the soil sample took 3 min to reach 38°C) (time A) and a constant period of 15 min (time B). Time A was previously determined in a parallel set of flasks using a thermometer with its bulb buried in the soil and ranged from 3 to 5 min irrespective of the temperature and soil type. After the heat shocks, the flasks were cooled to room temperature and incubated at 25°C. Flasks were covered with plastic film pierced with a needle to minimize the water loss during the incubation. Every 3 days, the soil water content was adjusted to its initial content (60% WHC).

Determination of Biological Properties

Soil respiration rates were monitored at 16, 30, 40, and 72 h after heat shocks. Flasks were capped with rubber septa and the air in the overhead space sampled from four replicates of each temperature treatment. CO_2 was determined using a gas chromatograph equipped with a thermal conductivity detector (Carle Series 100 AGC, Loveland, CO, USA).

The effect of heat shock on the microbial community structure was measured by analyzing the PLFA patterns at 3 and 30 days after disturbances. The first sampling date (day 3) corresponded to the time taken for respiration to reestablish at a stable rate, after the initial flush of CO_2. We assumed that the PLFA content of cells killed by the treatments would have been turned over during the initial CO_2 flush and that PLFA profiles represented the composition of the resistant viable microbial biomass that survived the heat shock. After homogenization, 3 g of soil were sampled from each of four replicates per treatment. Quantitative extraction procedures and reagents for the PLFA analysis were similar to those described by Butler et al. [9]. The PLFA composition of extracts was determined with an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a 25-m HP Ultra-2 column (internal diameter, 0.2 mm; film thickness, 0.33 μm) and a flame ionization detector (FID-GC). The identification of fatty acid methyl esters (FAMEs) was based on comparison with chromatograms of a mixture of 37 FAMEs (FAME 37 47885-4; Supelco, Bellefonte, PA, USA), a mixture of 24 bacterial FAMEs (P-BAME 24 47080-U; Supelco), 16:0 10-Me, and 18:0 10-Me (Matreya, Pleasant Gap, PA, USA), and 20:4 ω6c (Supelco). A standard curve prepared from a solution of tridecanoic acid methyl ester (Supelco) was used to convert chromatographic areas into picomoles of FAMEs. Profile composition was expressed in mole percent of PLFAs after standardizing the absolute amounts of individual PLFAs within each sample.

The summed mole percent values of FAMEs reported as typical of fungi, gram-negative bacteria, gram-positive bacteria, actinomycetes, and arbuscular mycorrhizal fungi (AMF) were used as signatures for these microbial groups [18, 42, 50, 52]. The sum of all FAMEs (picomoles per gram of soil) was used as an estimate of microbial biomass (FAME-MB).

Three soil enzymes were analyzed in the soil remaining in the flasks. Hydrolysis of FDA was determined by incubating soil samples for 2 h (37°C) and determining fluorescein released according to Schnürer and Rosswall [44] and modified by Dick et al. [15]. This assay evaluates the overall activity of proteases, lipases, and esterases [20, 24]. Activity of cellulase was determined by incubating 1 g of moist soil at 50°C for 24 h with 10 mL of 2 M acetate buffer (pH 5.5) containing carboxymethyl cellulose sodium salt (0.7% *w/v*). The reducing sugars released were determined with alkaline potassium hexacyanoferrate (III) at 690 nm [43]. Analytical triplicates were used for each soil sample. A mixture of soil and buffer without substrate was prepared as a blank for each sample. Laccase was extracted from soil samples according to Lang et al. [31] and its activity was measured by the oxidation of 2,2′-azino-di(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [37].

Data Analysis

Patterns of community composition represented by the relative abundance of microbially derived PLFA were graphically displayed using nonmetric multidimensional scaling (NMS) [30, 34] and Sørensen distance [6]. NMS run was based on a matrix that consisted of 80 soil samples (5 heat shocks×2 soil types×2 sampling periods×4 replicates) and 28 FAMEs using the PC-ORD statistical package, V4 (MjM Software, Gleneden Beach, OR, USA). The 28 FAMEs only included peaks in the region between tetradecanoic methyl ester (14:0) and arachidonic acid (20:4ω6c). FAMEs that occurred in less than 5% of all samples were excluded from the data matrix.

To simplify the ordination display, average NMS scores for each treatment were plotted. Statistical differences in the

microbial community composition were analyzed by sum of squares multivariate regression tree (SS-MRT) models [11]. The scores from the two NMS axes were used as response variables and soil type, heat shock, and sampling date were used as explanatory variables. A series of 20 tenfold cross-validations was run to choose the model tree size with minimum error rate [7, 12]. Because these trees were generally too large to interpret, the 1-SE rule was used, and the best tree was considered to be the smallest tree with an estimated error within one standard error of the minimum [7]. A library of sum of squares univariate regression tree routines (T. Therneau, unpublished data), extended by the inclusion of additional C routines to fit multivariate regression trees [11], was used in S-Plus 2000 (Insightful, Seattle, WA, USA) for the SS-MRT analysis.

Separate NMS ordinations were performed with PLFA data from each soil type to characterize changes in microbial communities using overlay gradients of microbial groups. This allowed us to evaluate the relative changes in microbial composition due to treatments without interference from the original microbial composition of each soil type.

The values of enzymatic activity, soil respiration, and FAME-MB for all heat shock-treated soils were transformed according to the following equation, as to express the percentage of change relative to the respective control in each sampling period:

$$\text{relative change } (\%) = \left[\left(\frac{T_i}{C_i}\right) - 1\right] \times 100$$

where T_i is the variable value in the treated soil sample at sampling time i and C_i is the average of the values obtained for the four control replicates of each sampling time i. The transformed values were used to evaluate the stability (resistance and resilience) of the two soils using the linear regression procedure (S-Plus) with soil type, sampling time, and heat shock temperature as explanatory variables. When the relationships between the variables and heat shock levels were not linear, a three-way analysis of variance was used instead. To evaluate the effect of each heat shock temperature in each soil relative to the controls, the untransformed values of each variable were submitted to a two-sided multiple comparison with a control (MCC) test (S-Plus).

Results

Characterization of the Forest and Agricultural Soils

Several physical and chemical properties differed between FST and AGR soils. Fourteen years of cultivation had decreased TOC (−37%), particulate organic C (−39%), and WSA (−13%) in the AGR soil relative to the FST soil (Table 1). Although the AGR soil had a lower pH and smaller aggregate mean diameter than FST soil, these differences were less remarkable.

Among the biological properties, the AGR soil expressed lower respiration (−40%), FAME-MB (−41%), and activities of laccase (−44%), cellulase (−20%), and hydrolysis of FDA

Table 1 Physical, chemical, and biological properties of soil samples (0–5 cm depth) collected under a tropical forest fragment and an adjacent no-till corn field in a Typic Fragiudult from Northeastern Brazil

Soil variables	Forest site	Agricultural site
Physical		
Texture	Sandy loam	Sandy loam
Aggregate weighed mean diameter (mm)	0.88±0.13	0.72±0.10
Water stable aggregate (%)	96.7±3.0	84.2±4.0
Chemical		
pH (H_2O)	5.45±0.17	5.19±0.15
TOC (%)	1.64±0.26	1.03±0.06
Carbon from particulate organic matter (%)	0.31±0.01	0.19±0.03
Biological[a]		
Soil respiration (μg C–CO_2 g^{-1} h^{-1})	0.25±0.02	0.15±0.01
Laccase activity (nmol ABTS azine cation g^{-1} min^{-1})	6.14±1.20	3.41±0.48
Cellulase activity (μg glucose g^{-1} h^{-1})	127.5±13.9	102.9±13.7
FDA (μg fluorescein g^{-1} h^{-1})	35.93±1.67	29.57±3.32
FAME-MB (nmol total FAME g^{-1})	30.2±5.40	19.6±5.70
Gram-positive bacteria (mol%)	28.94±0.42	29.87±2.50
Gram-negative bacteria (mol%)	6.54±0.09	4.24±0.36
Actinomycetes (mol%)	8.99±0.34	9.75±0.36
Fungi (mol%)	1.04±0.28	1.47±0.28
AMF (mol%)	3.19±0.04	2.78±0.15

Values are means for four replicates±standard deviation. Microbial group markers: gram-positive (15:0i; 15:0a; 16:0i; 17:0i; 17:0a); gram-negative (18:1ω7c; 17:0cy; 19:0cy); actinomycetes (16:0 10-Me; 17:0 10-Me; 18:0 10-Me); fungi (18:2ω6c); AMF (16:1ω5c)

[a] Biological properties were analyzed after the preincubation of soil samples for 10 days at 25°C and 60% of WHC

(−18%) (Table 1). The two soils also contained different microbial community structures based on PLFA profiles. Accordingly, the microbial community structure of the FST soil was richer in the PLFA markers for AMF (16:1ω5c) and gram-negative bacteria (18:1ω7c; 17:0cy; 19:0cy), whereas the AGR soil was richer in PLFA markers for actinomycetes (16:0 10-Me; 17:0 10-Me; 18:0 10-Me) and fungi (18:2ω6c).

Soil Respiration and Microbial Biomass

Heat shocks affected short-term respiration rates in both soils (Fig. 1). Sixteen hours after the treatments, FST soil samples treated at 50°C, 60°C, and 70°C showed significant increases in respiration above the unheated control soil of about 50%, 230%, and 430%, respectively. These same temperature treatments increased the respiration rates in AGR soil samples by about 50%, 150%, and 540%, respectively, relative to the unheated control. The respiration rates of soils treated at 40°C did not differ from the controls 16 h after the heat shock, but the rate was about 30% higher in the AGR soil 28 h after the heat shock. Despite the elevated flushes of CO_2 72 h after the heat shocks, the respiration rates of all treated soils were equal to or less than the controls (Fig. 1).

Heat shock treatments affected FAME-MB to a similar degree in both soils (Fig. 2). Although FAME-MB in soils treated at 40°C and 50°C were not significantly different from controls, samples treated at 60°C and 70°C decreased by 24% (SE=7%). However, it is important to notice that FAME-MB values obtained 3 days after the heat shocks may not correspond to the minimum biomass values obtained immediately after the heat shocks, as new microbial biomass could have been resynthesized from C released upon microbial death over this period.

Thirty days after the heat shocks, FAME-MB in the AGR soil remained at the same level found 3 days after disturbance, irrespective of heat shock level. In contrast, FAME-MB from the FST soil was drastically decreased by 76% (SE=13%) relative to the control in samples treated at 60°C and 70°C.

Stability of Soil Enzymes

To compare the functional stability (resistance and resilience) of AGR and FST soils, we evaluated FDA hydrolysis, cellulase activity, and laccase activity 3 and 30 days after the heat-induced microbial biomass reduction. Resistance was evaluated as the percentage of change in heat shock-treated samples relative to the control at day3. Both laccase and cellulase activities showed similar resistance pattern between the two soils (Fig. 3a, b); however, these activities differed in their degree of sensitivity to the heat shocks. For instance, the lower temperature (40°C) caused decreases of 19% and 11% in cellulase and laccase activities, respectively. The activities of both enzymes declined at the same rate (11%) in response to further incremental increases of 10°C in temperature up to 70°C.

By contrast, the FDA activity was more resistant in FST than in AGR soil. Although the activity in the AGR soil was unaffected by 40°C, this same treatment increased the activity in the FST soil by 5% ($p<0.05$) (Fig. 3c) and this difference persisted throughout the temperature range evaluated. Heat shock above 40°C significantly affected the FDA activities in both soils, which were reduced by 18% for each 10°C increment of temperature change.

The functional resilience of the two soils was assessed by evaluating the shift in enzyme activities toward the predisturbance condition between 3 and 30 days. Each enzyme had a unique resilience pattern, which depended on soil type. The FDA activities of the FST and AGR soils showed similar resilience patterns with the percent change between 3 to 30 days being equivalent regardless of temperature (Fig. 3c). However, this resilience was only expressed in response to the two highest heat shock

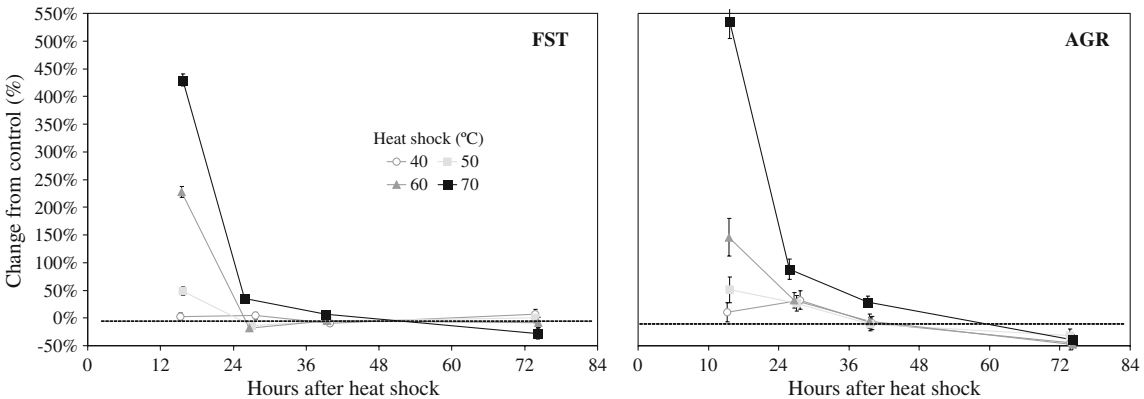

Figure 1 Relative changes in the rate of soil respiration up to 3 days after application of heat shocks. *Vertical bars* indicate the standard error ($n=4$)

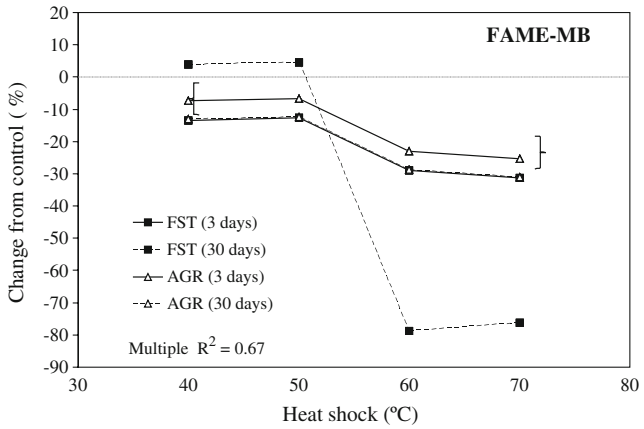

Figure 2 Percentage of change relative to control soils for FAME-MB along the gradient of heat shock temperatures. Forest soils (*FST*) are represented by *filled squares* and agriculture soils (*AGR*) by *open triangles*. *Full* and *dashed lines* correspond to samples taken at 3 and 30 days after heat shocks, respectively. *Lines within the same brackets* did not differ at $p<0.05$

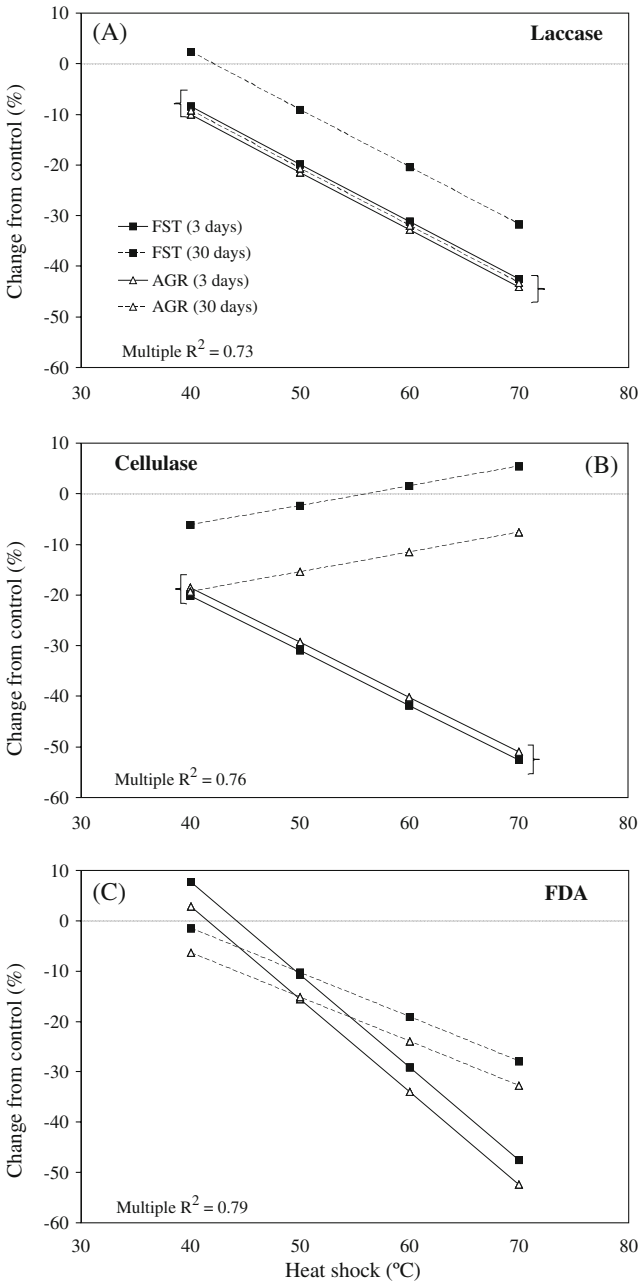

Figure 3 Fitted linear regression models for the percentage of change in soil enzymatic activities relative to controls along the gradient of heat shock temperatures. Forest soils (*FST*) are represented by *filled squares* and agriculture soils (*AGR*) by *open triangles*. *Full* and *dashed lines* correspond to samples taken at 3 and 30 days after heat shocks, respectively. *Regression lines within the same brackets* did not differ at $p<0.05$

temperatures (60°C and 70°C). Laccase activity was resilient only in the FST soil where the enzyme activity significantly shifted toward its predisturbance level between 3 and 30 days (Fig. 3a).Cellulase was the most resilient of the three enzymes especially in the FST soil (Fig. 3b). Thirty days after the heat shocks, the cellulase activity in the FST soil treated at 40°C was only 6% lower ($p<0.05$) than the controls; a recovery of almost 70% of the relative decrease observed at day 3. At higher temperatures, the recovery was even higher and inversely proportional to the loss of activity that occurred in response to the heat shocks. Cellulase resilience in the AGR soil was expressed only in samples treated above 50°C. The difference in the percentage of relative change between the two soils was equal to 13% and remained constant throughout the temperature range.

Stability of Microbial Community

The NMS ordination of sample units in the FAME space graphically illustrated the microbial community profiles at 3 and 30 days after the heat shocks (Fig. 4). About 87% of the total variability in PLFA profiles was represented by a 2-D NMS plot (64% along axis 1, and 23% along axis 2). Statistical differences among treatments were defined by the SS-MRT diagram (Fig. 5) which resulted in five groups (terminal nodes [TN]) represented in the NMS plot by encircling treatments with similar microbial communities. Three days after the heat shocks, the structures of the microbial communities from both soils were not significantly altered by any of the heat shocks (Fig. 4). However, changes were revealed in FST soil samples treated at 60°C and 70°C after 30 days of incubation. The overlay of microbial groups in the ordination of FST soil sample units revealed that changes in samples treated at 60°C and 70°C were mostly associated with decreases in AMF, fungi, and gram-negative bacteria (Fig. 6). The 30-day incubation also altered the microbial community in the AGR soil, but no significant differences were observed between the heat shock-treated soils and the controls on the same sampling date (Fig. 4). The overlay of microbial groups in the

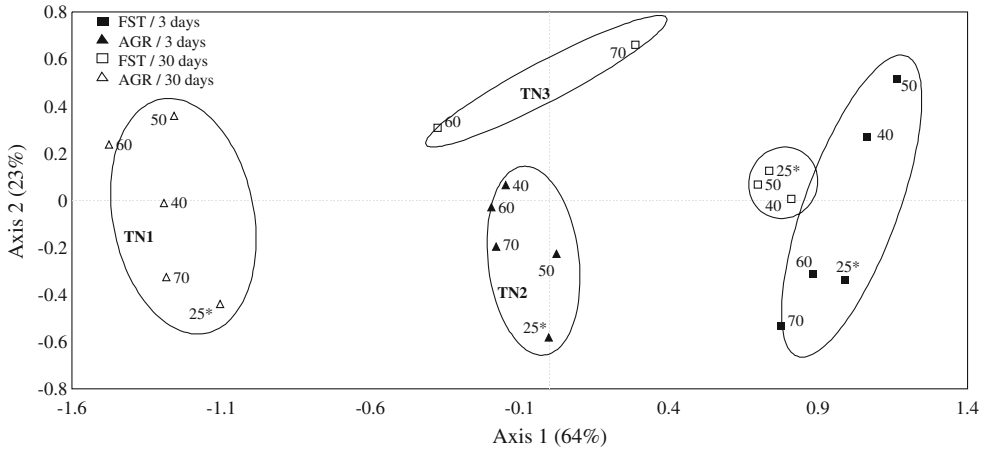

Figure 4 NMS ordination of PLFA profiles extracted from forest (*FST*) and agriculture (*AGR*) soils treated by heat shocks. Each observation plotted represents the average of four replicates. Heat shock temperature (in degrees Celsius) is indicated adjacent to the graph markers. Controls within each combination of soil type and sampling date are indicated by *asterisks*. Samples within the same *ellipse* do not differ significantly in their PLFA profiles according to the multivariate regression tree diagram (Fig. 5)

ordination of AGR soil sample units showed there were relative increase of actinomycetes and decreases of AMF and, at a lower intensity, of fungi, gram-positive, and gram-negative bacteria over the 30-day incubation (Fig. 6).

Discussion

Fourteen years of cultivation of the AGR soil caused degradation of several soil properties as indicated by decreases in total and particulate soil organic carbon, aggregate stability, aggregate weighed mean diameter, FAME-MB, and enzyme activity relative to the forest soil counterpart (FST) (Table 1). These results corroborate other studies that have demonstrated similar changes in soil properties following deforestation and soil cultivation [3, 27, 40, 46, 47]. In addition, cultivation altered the soil microbial community composition (Table 1), a phenomenon that has also been reported in tropical soils after forest conversion to agriculture [3, 49] or to cultivated pastures [2, 41]. In the following sections, we discuss how these changes in the AGR soil properties altered the microbial community and its functional stability.

Soil Microbial Functional Stability

Our results demonstrated a higher functional stability in the undisturbed FST soil compared with the adjacent AGR soil. This was indicated by the responses of the three enzyme activities, which were less affected (higher resistance) or recovered more quickly (higher resilience) in the FST soil after the heat shocks. For instance, laccase activity in the AGR soil did not show resilience to any temperature level, whereas there was a significant recovery in the FST soil (Fig. 3a). In the case of cellulase, both soils expressed resilience, but recovery was significantly more pronounced in the FST soil (Fig. 3b). The rate of return of cellulase activity was directly proportional to the magnitude of the

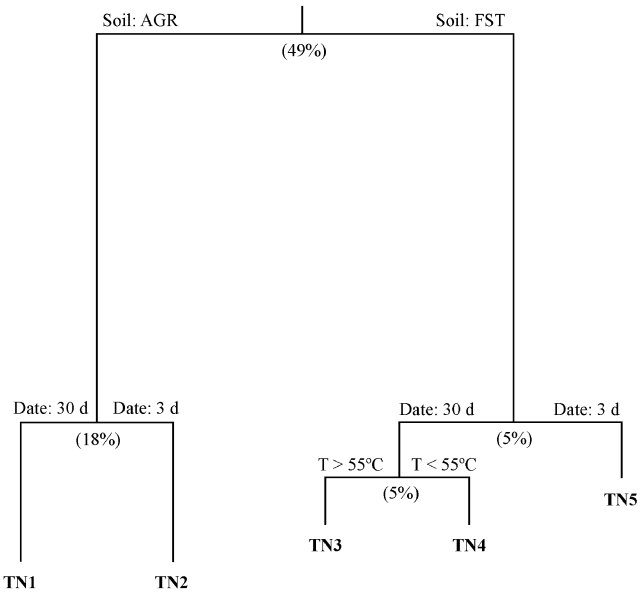

Figure 5 SS-MRT diagram showing the separation of heat shock-treated soils based on the first and second axes from the NMS ordination (Fig. 4). The tree model is based on successive dichotomic partitions of treatments according to similarities between their fatty acid profiles. The length of the vertical branches is proportional to the fraction of the total variance explained by the respective partition (values in *parenthesis* represent the percentage of variance explained in each split). Similar treatment groups are described above the tree branches after each partition. The diagram shows five TN which defined differences in microbial community composition in the heat shock-treated soils

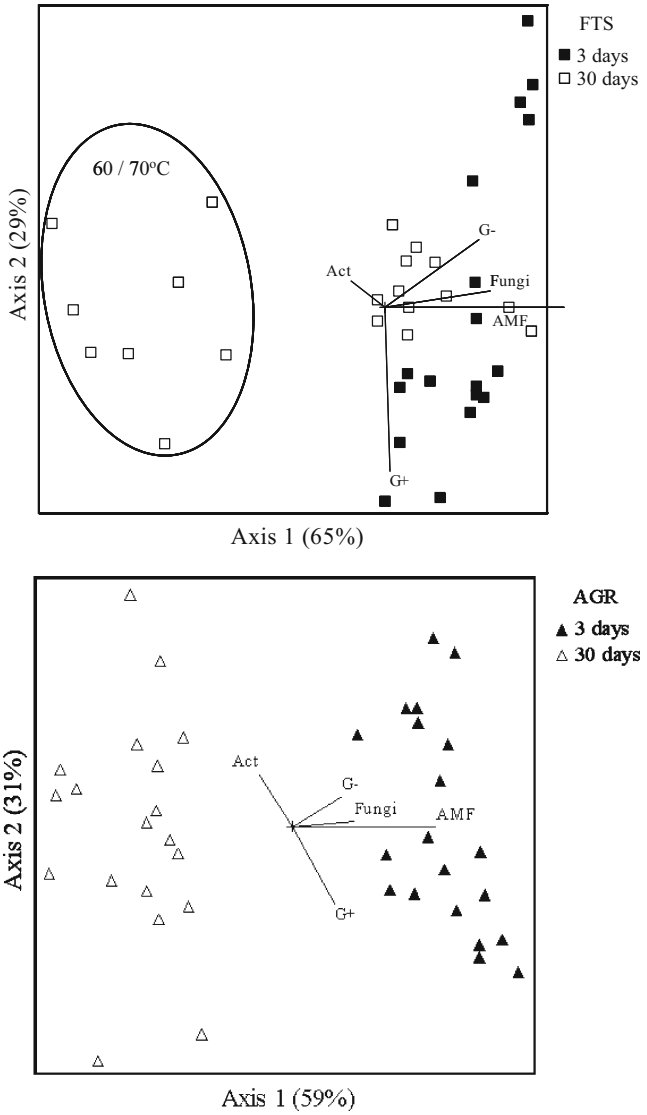

Figure 6 NMS ordination of PLFA profiles extracted separately for forest (*FST*) and agriculture (*AGR*) soils treated by heat shocks. Ordinations were rotated in order to maximize the correlation of axis 1 with sampling date. Major gradients of microbial groups were overlaid. *G−* gram-negative bacteria, *G+* gram-positive bacteria, *AMF* arbuscular mycorrhizal fungi, *Act* actinomycetes

heat shock and agrees with other studies that showed an induction of cellulase activity in response to different stress/disturbance factors (temperature, mineral salts, drying–rewetting, and combinations of these factors) in peat bogs [39, 45].

In contrast to laccase and cellulase, FDA hydrolysis differed between soils in terms of its resistance to heat rather than its recovery rate (Fig. 3c). The higher resistance of FDA hydrolysis activity in the FST soil might be associated with the high diversity of enzymes expressing this type of catalytic activity. It is also possible that thermal stability might reflect the protective effect of organic substances and the formation of organic clay–enzyme complexes [26, 28]. In contrast to FDA hydrolysis, cellulase and laccase activities were similarly reduced in the two soils in response to heat shocks (Fig. 3a, b). This fact may be due to the differences between enzymes regarding their adsorption and complexation with soil colloids. For instance, it has been suggested that the major portion of exoglucanase and endoglucanase activity is found in soil solution or attached to the outer surfaces of cellulolytic microorganisms [25]. Therefore, if the cellulase complex does not form organocomplexes, the higher SOC in the FST soil may have had little or no effect on the stability of this enzyme. We have found no reports on the effect of soil organic colloids on the thermal stability of laccase; however, laccase adsorption to a mineral colloid (aluminum hydroxide) had little effect on its thermal stability within the 40–60°C range [1].

Although the resistance of enzymes to heat shocks might depend on physicochemical interactions with soil colloids, their resilience will depend on the survival or proliferation of microbial populations capable of producing new enzymes. The higher total SOC in the FST soil does not seem to be the reason for the higher resilience observed in this soil. Besides possessing higher SOC, the FST soil contained initially higher microbial biomass and enzymatic activity (Table 1), which means that, in order to show equal rates of resilience, this soil must produce more enzymes in comparison with the AGR one. The ratio between FAME-MB and total SOC calculated from Table 1 showed only a small difference between soils (18.4 for FST and 19.0 for AGR), which suggests that the relative availability of resources to sustain microbial activity was similar among soils. This observation indicates that differences in laccase and cellulase resiliencies among the two soils might be more associated with differences in microbial diversity and composition than in resource availability.

It has been proposed that the higher the microbial diversity and its inherent functional redundancy, the faster the rate at which a soil ecosystem can return to a prestress state [13, 48]. Therefore, the lack of resilience of laccase activity and the lower resilience of cellulase activity in the AGR soil compared to the FST soil suggest that the deforestation followed by soil cultivation at this site caused a reduction in microbial diversity. This fact would also explain the similar resilience pattern of FDA hydrolysis in both soils because it represents the activity of a "broad-scale" group of enzymes including proteases, lipases, and esterases [20, 24]. In contrast, cellulase and laccase activities are "narrow-niche" enzymes that represent more specific functions within the soil microbial community. Assuming an inverse relationship between the specificity of the enzyme and the number of microbial species able to produce them, it is less likely that a decrease in microbial diversity in the AGR soil would affect the resilience of

FDA hydrolysis compared to laccase and cellulase. This idea is corroborated by several studies that show lower stability of narrow-niche soil functions compared with broad-scale functions when microbial diversity was reduced. For example, decreasing microbial diversity by progressive fumigation of a high organic matter soil from a temperate climate either did not affect or increase microbial growth and decomposition of plant residues, but it did decrease more specific functions such as nitrification, denitrification, and methane oxidation [22]. In another study, the addition of copper sulfate or benzene to soils similar to that previous study with naturally differing levels of diversity (mineral and organomineral soils) did not affect the stability of a broad-scale function (i.e., mineralization of ^{14}C-labeled wheat shoot), but the resilience of the narrow-niche function (mineralization of ^{14}C-labeled 2,4-dichlorophenol) only persisted in the high diversity soil [19].

Microbial Community Composition

Microbial community composition from soils assessed 3 days after the heat shocks did not show significant differences relative to the controls (Fig. 4), suggesting that the microbial groups from each soil were equally sensitive to temperature. This observation suggests that the heat-induced biomass reduction method did not discriminate among different microbial groups. Hence, effects observed on functional stability might be restricted mainly to the original microbial composition.

After 30 days of incubation, the microbial community of the AGR soil shifted toward a relatively more actinomycetes-enriched and gram-positive-, gram-negative-, fungi-, and AMF-depleted community (Fig. 6). However, these changes were also observed in the control soils indicating that recovery incubation time, rather than heat shock per se, had the major influence on the microbial community composition of this soil. In contrast, microbial community composition of the FST soil samples treated at 40°C and 50°C were quite stable showing no shift in composition due to heat shock treatment or incubation time (Fig. 4). The same pattern was not observed in FST soil samples treated at 60°C and 70°C which had a significant shift in microbial composition between 3 and 30 days, mostly characterized by relative decreases in gram-negative bacteria, fungi, and AMF (Fig. 6). These differences suggest a threshold temperature between 50°C and 60°C above which representative microbial species from the community were eliminated due to their heat sensitivity, thus allowing other surviving species to establish a new community with a different structure. This is corroborated by the significant decrease in FAME-MB in FST soil samples treated at 60°C and 70°C, which reached levels up to 80% lower than the controls at the end of the incubation period (Fig. 2). Nevertheless, it is surprising that, regardless of these changes, these FST soil samples still showed resilience for all three enzymes and generally higher than that observed in the AGR soil where a smaller decline of biomass was observed. This fact indicates that the biomass that persisted in FST soil samples treated at 60°C and 70°C was more active than the original biomass. This increase in activity per unit of FAME-MB was probably induced by an increase in availability of labile nutrients released from the dead biomass as well as by the relative reduction of the competition for resources. Wright et al. [51] reported that the activity of bacterial populations grazed by *Colpoda steinii* was drastically increased relative to the ungrazed population, despite a significant decrease in bacterial biomass caused by grazing. The authors suggest that the activity of the large, ungrazed bacterial population was substrate-limited and that the release of nutrients from the preyed cells resulted in a flush of activity by the bacteria that escaped predation. By analogy, we might suggest that nutrients released from cells killed by the heat shocks would cause a similar flush in enzyme activity by the surviving microorganisms.

Conclusions

In the studied soil, change in land use from an undisturbed forest to long-term cultivation was associated with changes in soil properties and with the establishment of a new microbial community that was less functionally stable. The reduced resilience of laccase and cellulase in the AGR soil was probably the result of a limited diversity of microorganisms capable of producing these enzymes, rather than of the availability of resources. Moreover, the similar resilience of FDA hydrolysis activity in both FST and AGR soils as well as the lack of sensitivity of other broad-scale soil functions to stress/disturbance observed elsewhere corroborate the idea of a considerable functional redundancy of soil microorganisms. Conversely, the sensitivity of narrow-niche functions observed here and in other previously stressed/disturbed soils suggests that specific functional parameters are more sensitive measurements for evaluating change in the ecological stability of soils.

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