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Functional Diversity of Soil Bacterial Communities in the Tabonuco Forest: Interaction of Anthropogenic and Natural Disturbance[1]

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ABSTRACT

Anthropogenic and natural disturbances play critical roles in affecting the structure and function of Caribbean ecosystems, where hurricanes represent important disturbances superimposed on a landscape modified by human agricultural and forestry practices. Based on the differential catabolism of a suite of 128 carbon sources by soil bacterial communities, we focus on four aspects of functional diversity (total substrate activity, substrate richness, substrate evenness, substrate diversity) in the tabonuco forest of Puerto Rico, and assess the degree to which their spatial variability is a consequence of historical landuse or impacts of Hurricane Hugo. Considerable microspatial heterogeneity characterizes the functional diversity of forest soil communities, but the degree of hurricane damage to aboveground plant communities is positively related to all four indexes of functional diversity 5yr after the hurricane. No differences in functional diversity were detected with respect to historical landuse, after controlling for the effects of hurricane damage. However, this lack of significance may be an artifact because the spatial distribution of hurricane damage is not independent of historical landuse. As a consequence, contemporary studies of spatial heterogeneity that do not account for historical patterns of anthropogenic or natural disturbance may yield spurious or incorrect conclusions. Long-term studies help to rectify this problem and are especially important within the context of evaluating the impacts of increasing human demands on natural ecosystems.

RESUMEN

Las perturbaciones antropogénicas y naturales juegan un rol crítico al afectar la estructura y función de los ecosistemas caribeños, donde los huracanes representan perturbaciones importantes superpuestos a un paisaje modificado por prácticas agrícolas y forestales humanas. Basado en el catabolismo diferencial de un conjunto de 128 fuentes de carbono por comunidades de bacterias del suelo, centramos nuestro estudio en cuatro aspectos de la diversidad funcional (actividad total del substrato, riqueza del substrato, uniformidad del substrato, diversidad del substrato) en el bosque de tabonuco de Puerto Rico, y evaluamos el grado en que su variabilidad espacial es una consecuencia de la historia del uso de la tierra o de los impactos del Huracán Hugo. Una considerable heterogeneidad espacial caracteriza la diversidad funcional de las comunidades de suelo de bosque, pero el grado de daño del huracán a las comunidades de plantas sobre el suelo está positivamente relacionado a todos los cuatro índices de diversidad funcional cinco años después del huracán. No se detectaron diferencias en la diversidad funcional con respecto a la historia del uso de la tierra, después de controlar los efectos del daño del huracán. Sin embargo, esta ausencia puede ser un artefacto ya que la distribución de daño del huracán no es independiente a la historia del uso de la tierra. Como consecuencia, los estudios contemporáneos de heterogeneidad espacial que no tengan en cuenta los patrones históricos de disturbio antropogénico o natural pueden resultar en conclusiones falsas o incorrectas. Estudios a largo plazo ayudan a rectificar este problema y son especialmente importantes dentro del contexto de evaluar los impactos de las crecientes exigencias humanas sobre los ecosistemas naturales.

Key words: disturbance; diversity; hurricane; landuse; microbial communities.

DISTURBANCE IS A PERVASIVE THEME in contemporary ecology (Sousa 1985, Pickett & White 1985). Regardless of geographic or climatic characteristics, every region of the planet is affected by anthropogenic or natural disturbances. These effects can be of a structural or functional nature, and are manifest at the level of populations, communities, and ecosystems. Indeed, disturbances are one of the most important ecological forces that impart heterogeneity to the landscape (Kolasa & Pickett 1991).

The islands of the Caribbean represent ideal ecological theaters in which to examine the role of disturbance. Because of their isolation and distance from potential sources of colonization, they are less complex than mainland counterparts, at least from a taxonomic and trophic perspective. At the same time, hurricanes represent important disturbance agents of high intensity, large scale, but low fre-

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quency (Waide & Lugo 1992) that dominate the disturbance regime of many Caribbean landscapes. Continuing ecological investigations of the forest ecosystems of Puerto Rico provide a long-term context in which to examine the effects of such low frequency, but large scale disturbances (Walker *et al.* 1991). The Caribbean basin in general and Puerto Rico in particular, like much of the tropics, has been drastically altered as a consequence of anthropogenic disturbance related to agriculture (*e.g.*, sugar cane, coffee, and banana plantations) and forestry (*e.g.*, clear cutting, selective logging, and monospecific tree plantations). As a result, evaluation of the consequences of natural disturbances must be conducted while simultaneously assessing the effects of landuse history. One of the greatest challenges of landscape ecology is to understand the manner in which both natural and anthropogenic disturbance interact to affect the distribution and abundance of organisms.

Considerable attention has focused on the manner in which disturbances affect the population biology and community ecology of plants and animals, with scant attention devoted to microbial communities. Moreover, a much larger number of studies have examined changes in the structural attributes of ecosystems rather than functional responses, such as those involving production and decomposition. We present results addressing the impacts of anthropogenic (historic landuse) and natural (hurricane) disturbance on the functional diversity of soil bacterial communities.

BACKGROUND.—Microorganisms play a number of important roles as pathogens, symbionts and saprophytes. Nonetheless, basic ecological studies of microbial communities are constrained by a limited ability to identify and quantify taxa. A variety of methods have been employed to elucidate the taxonomy and abundances of microorganisms in ecological communities, including isolation and successive culturing on selective media (*e.g.*, Mills & Wassel 1980), determination of fatty acid methyl esterase profiles (Haack *et al.* 1994), evaluation of 16S rRNA sequences (Giovannoni *et al.* 1990) and quantification by DNA-DNA hybridization (Lee & Fuhrman 1991). Despite these efforts, taxonomic and ecological characteristics of microbial communities remain poorly understood.

Garland and Mills (1991) were among the first to demonstrate the utility of patterns of sole-source carbon substrate utilization as a means to distinguish among bacterial communities. They proposed that the identity, number and extent of catabolism of the 96 specific substrates on Biolog (Biolog Inc., 3938 Trust Way, Hayward, California 94545, U.S.A., 800-284-4949) microtitre plates provide physiological profiles that can be used to differentiate among communities. Although originally designed to help identify strains of bacteria, use of the Biolog system has been extended to screening isolates for their potential to degrade volatile environmental contaminants, such as toluene and xylene (Strong-Gunderson & Palumbo 1994), evaluating metabolic variation among strains of particular bacterial groups and isolates (Fredrickson *et al.* 1991; Knight *et al.* 1995), as well as comparing catabolic attributes of entire communities (Garland & Mills 1991, Winding 1993, Zak *et al.* 1994, Cox *et al.* 1995, Harrell *et al.* 1995).

From an ecological perspective, differences in the physiological or catabolic attributes of bacterial communities may provide as much insight into biotic responses to environmental variation as do analyses based on taxonomic structure. For example, principal components analysis (PCA) has been used to differentiate communities, based on data from Biolog plates (Garland & Mills 1991, Winding 1993, Zak *et al.* 1994). Moreover, Zak *et al.* (1994) calculated indexes of diversity, evenness, and richness from these data in much the same manner as is often applied to taxonomic data. Subsequent application of this approach to evaluations of aquatic ecosystems demonstrated relationships between indexes of functional diversity and surrounding landuse and attributes of water quality (Cox *et al.* 1995, Harrell *et al.* 1995). In a recent evaluation of the Biolog-based methodology, Haack *et al.* (1995) found that model communities constructed from pure strains of bacteria yielded reproducible patterns of substrate utilization. However, the identities and abundances of microorganisms were not correlated to the degree of substrate utilization. Clearly, complex interactions exist even within simple, model communities.

In the present study, we used the Biolog system to examine the effects of Hurricane Hugo and landuse history on the spatial patterns of soil bacterial communities in the lower montane rainforest of Puerto Rico. Our central hypothesis is that changes in soil bacterial communities occur in association with modification of biotic and abiotic features of the landscape that derive from natural and anthropogenic disturbance, and that these changes are

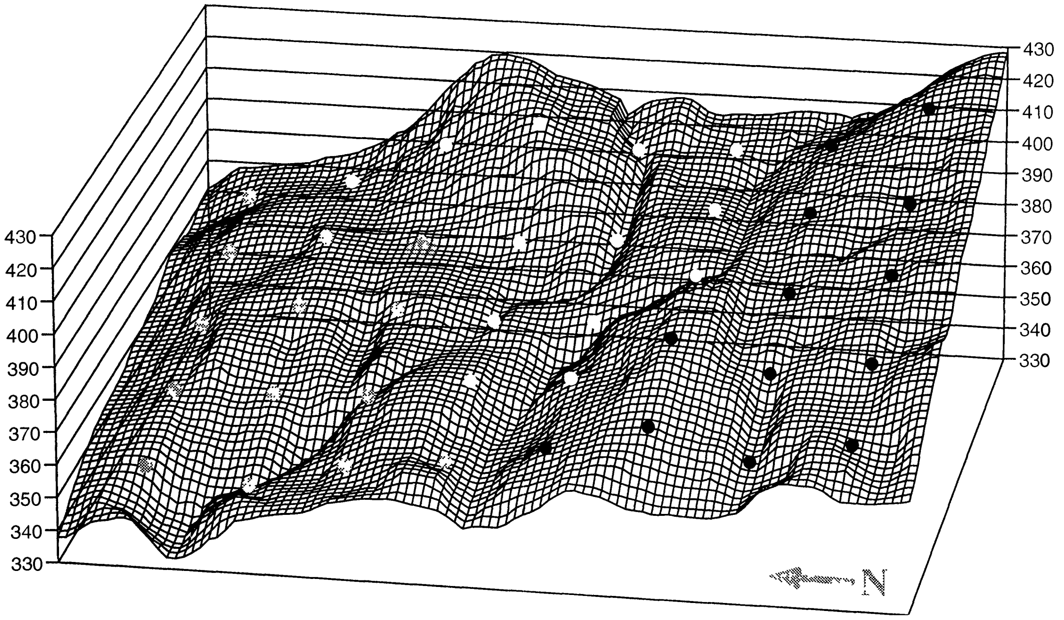

FIGURE 1. Schematic, three-dimensional representation of the elevational profile of the Hurricane Recovery Plot, with the location (circles) of the 40 sampling points and their landuse classification (white, intensively logged; grey, coffee plantations; black, light logging). Elevations are in meters above sea level.

manifested as site-specific differences in the physiological profiles of the communities.

MATERIALS AND METHODS

Study site.—The Luquillo Experimental Forest (LEF) is located in the northeast corner of Puerto Rico and comprises four life zones as a consequence of elevational changes in climate and soil characteristics. The Subtropical Montane Wet Forest (Holdridge System; Ewel & Whitmore 1973) is the most extensive life zone and occurs at elevations below 650 m. It represents approximately 70 percent of the landscape. Because it is dominated by *Dacryodes excelsa* (tabonuco), this life zone is referred to as the tabonuco forest. Other common trees include *Prestoea montana, Manilkara bidentata* and *Sloanea berteriana* (Odum & Pigeon 1970, Brown *et al.* 1983). Monthly temperatures are relatively invariant, ranging from 21° C in January to 25° C in September (Brown *et al.* 1983). Annual precipitation is considerable (mean ± standard error, 375.95 ± 79.47 cm) and slightly seasonal, with lower values from January to April (19.57 to 23.71 cm) and higher values (35.01 to 45.99 cm) in the remaining months (Brown *et al.* 1983). Soils are dominated by zarzal clays, deep ultisols of volcanic origin (Soil Survey Staff 1995).

This research was conducted in the tabonuco forest at El Verde Field Station (18° 10′ N, 65° 30′ W) on the Hurricane Recovery Plot (HRP), a 16 ha rectangular grid located on the west side of the LEF (Fig. 1). A concise statement of the disturbance history of the HRP related to hurricanes and anthropogenic activity appears in Zimmerman *et al.* (1994), with more comprehensive coverage of hurricane effects in Walker *et al.* (1991). A brief summary of this information follows.

Hurricane Hugo was the most recent (September 1989) hurricane to strongly affect the LEF. It produced considerable destruction to the flora, that is, of the 13,078 stems alive on the HRP prior to the hurricane, 16.7 percent experienced severe stem damage, 6.9 percent were uprooted, 7.5 percent had broken stems, 1.4 percent had root breaks, and 0.8 percent were bent. Similarly, the abundance and distribution of the fauna were altered drastically by the hurricane (Reagan 1991, Waide 1991, Willig & Camilo 1991, Woolbright 1991, Gannon & Willig 1994). Other than Hurricane Hugo, two destructive hurricanes (1928 and 1932) and two less severe hurricanes (1931 and 1956) struck the LEF in the last 75 years.

All of the vegetation on the HRP should be considered secondary forest. Forest cover in the southwestern portion of the grid was > 80 percent

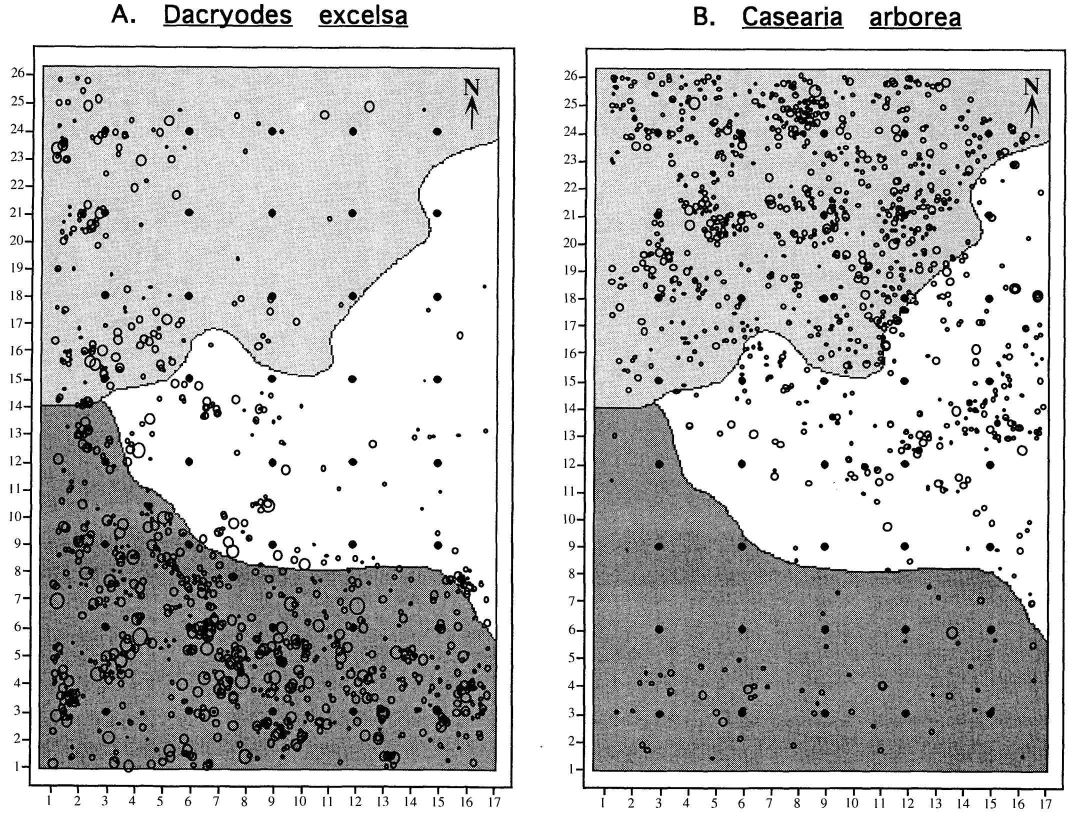

FIGURE 2. Diagramatic representation of the Hurricane Recovery Plot, indicating land use history (white, intensively logged; grey, coffee plantations; black, light logging), as well as the distribution of *D. excelsa* (A) and *C. arborea* (B). Solid dots represent the 40 sampling points. Open circles indicate the locations of individual trees; diameters are proportional to tree diameter at breast height.

in 1936 and considered to be similar to old growth stands of forest (*cf.* Odum 1970) prior to Hurricane Hugo. Other portions of the HRP were subject to clear cutting and other agricultural practices before its purchase by the USDA Forest Service in 1934. Forest cover on the northwestern portion of the grid in 1936 was between 50–80 percent, while the east-central portion of the grid had < 50 percent cover. Selective removal of tabonuco in 1937, 1944, 1946, and 1953 further reduced stand volume (Odum 1970). Immediately before Hurricane Hugo, the legacy of this landuse was reflected in the heterogeneous distribution of tree species on the grid. For example, tabonuco was abundant in the least disturbed area and quite rare on the more severely disturbed areas (Fig. 2a). Conversely, *Casearia arborea* was quite rare on portions of the HRP that were least modified by landuse, and abundant on more disturbed portions of the grid (Fig. 2b).

DISTURBANCE CRITERIA.—Based on structure of the canopy in 1936 aerial photographs, we classified each site (sampling point) of the HRP into one of three landuse categories: 1) previous coffee plantations (moderate disturbance; 50–80% cover), 2) intensive logging activities (high disturbance; < 50% cover), and 3) light logging activities (low disturbance; > 80% cover). In this study, we obtained 14 soil samples from the coffee plantation area, 13 from intensive logging sites, and 13 from sites with light logging. Eight sites were replicate-sampled for an assessment of within-site, microspatial variation, of which three occurred within each of the high and moderate cover categories and two were located within the low cover category.

The impact of recent natural disturbance on each of the 40 sites was assessed by two estimates of the degree to which the previously closed canopy was disrupted by Hurricane Hugo. For each site, a spherical densiometer reading was taken in each of the four cardinal directions between 27–28 July 1991. The mean of these readings represented canopy openness. Similarly, the amount of light penetrating to a level approximately 1.5 m from the forest floor was measured at each site using a ceptometer (December 1990–March 1991). Readings were restricted to clear days between 1200 and 1400 h. We did not have direct measures of canopy openness or disturbance immediately after the hurricane. Subsequent recovery or other disturbances unrelated to Hurricane Hugo but prior to 1991 could have affected measures of openness that we attribute to the hurricane. Nonetheless, the direction of such mitigating effects is unclear and we have no reason, *a priori,* to expect them to bias analytical results.

SAMPLE PROCESSING.—Soil samples were collected within a 4 hr period (7 August 1994) from each of 40 sites on the HRP (one sample per site; Fig. 1). These samples were used to assess landscape variation associated with landuse or disturbance. Four additional samples were collected from eight of these sites to assess the magnitude of microspatial variation within sites. At each of these eight sites, a sample was obtained from the center point and at a distance of 0.5 m from the center point in each of the cardinal directions, for a total of five replicates per site. In all cases, soil samples were taken to a depth of 15 cm with a 2.5 cm diameter soil corer. Soils were transported to the laboratory and activities of bacterial communities examined within 36 h of collection. Although collection sites were evenly distributed across the landscape based on the grid system (*i.e.,* hyperdispersed), such deviations from random sampling likely have little impact on the validity of subsequent statistical analyses (Ludwig & Reynolds 1988).

Standard dilutions were prepared from 10 g (dry mass) soil aliquots to achieve a final dilution of 10^{-4}. A 1 min blending at high speed in 0.2 percent water agar was conducted in the initial dilution to ensure homogeneous dispersion of the soil particles. A 150 μl aliquot of the final dilution was added to each of the 96 wells on both Gram (+) and Gram (−) Biolog microplates. Inoculated microplates were incubated at 25° C and absorbance at 590 nm (peak absorbance of tetrazolium dye) was examined every 12 h from 24 to 72 h. Each Biolog plate has a well that contains no substrate (a control); the absorbance of this well was subtracted from the absorbances of all others on the plate. Readings did not extend beyond 72 h because color development begins to occur in control wells at this time, apparently from bacteria using dissolved organic material contained in the inoculum; fungal growth becomes apparent at this time as well (Zak *et al.* 1994). For the substrates that appear on both Gram (+) and Gram (−) plates, only the higher of the two absorbances was used in subsequent analyses.

QUANTITATIVE ASSESSMENTS.—The Biolog system quantifies the utilization of specific carbon compounds by bacterial communities inoculated on Gram (−) and Gram (+) microplates (Biolog 1993). By combining the two types of microplates in the analysis, 128 unique carbon compounds (Table 1) can be used to evaluate the physiological profiles of these communities. The degree to which a particular substrate is utilized is quantified by measuring the intensity of color change caused by incorporation of tetrazolium dye into respiring bacteria. Thus, the functional attributes of the community are revealed by the particular pattern of catabolic activities with respect to this suite of substrates (Bochner 1989). The number and categories of utilized substrates, as well as activities, constitute a data set from which we assess functional diversity. Color development in each well reflects species composition, density, activity and physiological state of taxa contained in the inoculum. We do not maintain that functional diversity is strongly correlated with taxonomic diversity, although it may be so in some cases. Rather, we contend that functional diversity is intrinsically interesting as an alternative aspect of ecological systems.

We examined functional diversity of these communities in a quantitative manner that is analogous to treatments of taxonomic diversity. At sites on the HRP, functional diversity of the bacterial community was characterized by four indexes (Zak *et al.* 1994): total substrate activity, substrate richness, substrate evenness, and substrate diversity. The simplest of these measures is substrate richness (S), the number of different substrates that are used by the bacterial community. Substrate diversity (H') includes both substrate richness and substrate evenness, and was quantified by the Shannon-Weiner Index (Magurran 1988) as:

$$H' = -\Sigma\, p_i(\ln p_i)$$

where p_i is the ratio of the activity on a particular substrate to the sum of activities on all substrates. Similarly, substrate evenness (E) measures the equitability of activities across all utilized substrates, and is defined by:

$$E = H'/H'_{max} = H'/\ln S$$

TABLE 1. *Carbon substrates included in the Gram (−) and Gram (+) Biolog microplates categorized by substrate guilds.*

Carbohydrates	Carboxylic Acids	Amino Acids
3-methyl glucose	acetic acid	D,L-carnitine
adonitol	α-hydroxybutyric acid	D-alanine
α-D-glucose	α-ketobutyric acid	D-serine
α-D-lactose	α-ketoglutaric acid	gamma-aminobutyric acid
α-methyl-D-galactoside	α-ketovaleric acid	glycyl-L-aspartic acid
α-methyl-D-glucoside	β-hydroxybutyric acid	glycyl-L-glutamic acid
α-methyl-D-mannoside	cis-aconitic acid	hydroxy-L-proline
arbutin	citric acid	L-alanine
β-methyl-D-galactoside	D,L-lactic acid	L-alanyl-glycine
β-methyl-D-glucoside	D-galactonic acid lactone	L-asparagine
cellobiose	D-galacturonic acid	L-aspartic acid
D-arabitol	D-gluconic acid	L-glutamic acid
D-fructose	D-glucosaminic acid	L-histidine
D-galactose	D-glucuronic acid	L-leucine
D-mannitol	D-malic acid	L-ornithine
D-mannose	D-saccharic acid	L-phenylalanine
D-melezitose	formic acid	L-proline
D-meliobiose	gamma-hydroxybutyric acid	L-pyroglutamic acid
D-psicose	itaconic acid	L-serine
D-raffinose	L-lactic acid	L-threonine
D-ribose	L-malic acid	
D-sorbitol	malonic acid	*Miscellaneous*
D-tagatose	N-acetyl-L-glutamic acid	2,-deoxyadenosine
D-trehalose	p-hydroxy-phenylacetic acid	2,3-butanediol
D-xylose	propionic acid	adenosine
gentiobiose	pyruvic acid	adenosine-5-monophosphate
i-erythritol	quinic acid	amygdalin
L-arabinose	sebacic acid	bromo-succinic acid
L-fucose	succinic acid	D,L-alpha-glycerol phosphate
L-rhamnose		D-lactic acid methyl ester
lactulose	*Polymers*	fructose-6-phosphate
m-inositol	α-cyclodextrin	glucose-1-phosphate
maltose	β-cyclodextrin	glucose-6-phosphate
maltotriose	dextrin	glycerol
mannan	glycogen	inosine
methyl pyruvate	inulin	salicin
mono-methyl succinate	tween 40	thymidine
N-acetyl-D-galactosamine	tween 80	thymidine-5-monophosphate
N-acetyl-D-glucosamine		uridine
N-acetyl-D-mannosamine	*Amines/Amides*	uridine-5-monophosphate
palatinose	2-aminoethanol	urocanic acid
sedoheptulosan	alaninamide	
stachyose	glucuronamide	
sucrose	lactamide	
turanose	phenyl-ethylamine	
xylitol	putrescine	
	succinamic acid	

Total substrate activity is simply the sum of all readings of the different substrates.

Each of these indexes has a temporal dimension that emerges during incubation (Fig. 3); we estimated each component of functional diversity at each of the five incubation times (24, 36, 48, 60 and 72 h) for the entire suite of substrates. We contend that early readings are closer representations of bacterial functions in soils at the time of sampling, while later readings likely reflect the functional possibilities of these soil communities. Thus, the resulting temporal development of substrate utilization may provide insights to differences in both current and potential functionality of soil communities, as related to disturbance. In addition, this set of substrates can be divided into six guilds or groups of chemically-related compounds (Table 1). We also estimated each index at each time for each of the six guilds.

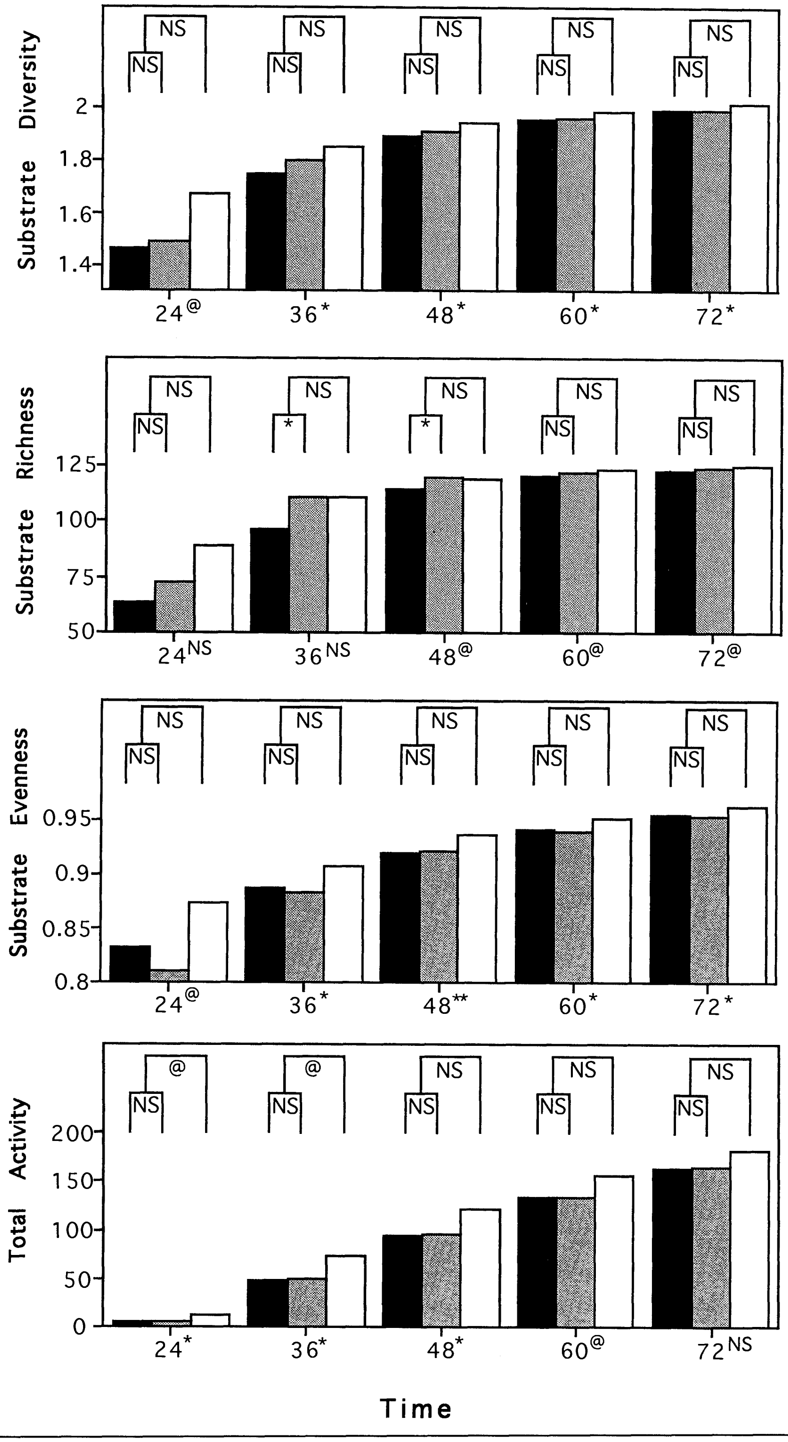

FIGURE 3. Histograms representing mean values for indexes of microbial functional diversity, classified by historical landuse (white, intensively logged; grey, coffee plantations; black, light logging) on the Hurricane Recovery Plot. Superscripts associated with inoculation times indicate the significance of covariates (hurricane-induced effects). *A priori* contrasts are indicated by dendrograms above each histogram; their significance is indicated at levels $0.001 < P \leq 0.01$ (**), $0.01 < P \leq 0.05$ (*), $0.05 < P \leq 0.10$ (@), and $P > 0.10$ (NS). Time is hours of incubation.

STATISTICAL ANALYSES.—We assessed microspatial variation for each index of bacterial functional diversity by conducting a two-level, nested ANOVA (Sokal & Rohlf 1995). Nesting categories were microsites within sites ($N = 5$) and sites ($a = 2$ or 3) within landuse categories. The effects of landuse history and hurricane-induced disturbance were assessed by an analysis of covariance (ANCOVA) for each of the indexes of bacterial functional diversity, with landuse classification as a categorical factor, and mean densiometer reading and ceptometer reading as covariates. All possible contrasts were evaluated using *a posteriori* Student-Newman-Keuls tests on residuals from the ANCOVA. More powerful, orthogonal, *a priori* contrasts of the adjusted means from the ANCOVA were used to compare the most disturbed sites to the moderately disturbed sites, as well as the least disturbed sites to all others. Analyses were conducted at each inoculation time for all substrates, as well as for each of the six guilds, separately. A multivariate analysis of variance (MANOVA) with mean densiometer and ceptometer readings as dependent variables and landuse as a categorical factor was conducted to assess if damage from Hurricane Hugo was related to prior landuse.

All statistical analyses (Sokal & Rohlf 1995) were conducted with experiment-wise error rate set at 0.05 using SPSSX (SPSS 1990). Because our analyses seek patterns and are based on relatively few sites ($N = 40$), we report differences as approaching significance ($0.10 \geq P > 0.05$), as well as being significant ($P \leq 0.05$).

RESULTS AND DISCUSSION

MICROSITE VARIATION.—For all substrates combined, regardless of guild, variation among sites within landuse categories did not contribute significantly to the overall variation in substrate richness, substrate evenness, substrate diversity, or total substrate activity. The only exceptions to this pattern involved substrate diversity ($P = 0.017$) and substrate evenness ($P = 0.004$) at 24 h incubation. At the guild level, variation among sites within landuse categories also contributed little to overall variation. Of the 20 analyses conducted for all combinations of diversity index (4) and time (5) for each guild, most were not significant. Miscellaneous compounds were the exception in that variation among sites within damage categories was significant in 7 of 20 analyses (diversity at 36 and 48 h incubation; richness at 60 h; evenness at 24, 36 and 48 h; total activity at 24 h). At least 67 percent of the total variance in an index was a consequence of microspatial differences for all substrates combined, regardless of guild. A similar proportion of the total variation in an index was a result of microspatial variation when analyses were restricted separately to each of the six guilds.

Clearly, considerable microspatial variation characterizes the functional diversity of soil bacterial communities in the tabonuco forest. In fact, variation in catabolic activity within sites (circles of 1 m diameter) was greater than the variation among sites within landuse categories. Microgeographic variation in soil temperature, water content, organic matter characteristics and nutrient pools likely influence the taxonomic composition of bacterial assemblages, as well as their density and physiological state. Aboveground differences in the flora affect litter inputs (qualitatively, quantitatively and spatially) as well as the carbon and nutrient inputs in the rhizosphere. Such biotic and abiotic heterogeneity influences the variability in bacterial floras, and thus, would be expected to affect their functional attributes. In a different context, this has been documented by Haack *et al.* (1995) and others (*e.g.*, Garland & Mills 1991).

ANALYSES BASED ON TOTAL SUITE OF SUBSTRATES.—In general, the two covariates (average densiometer and ceptometer readings) that assess disturbance related to Hurricane Hugo, together accounted for a significant amount of variation in all four indexes (substrate richness, substrate diversity, substrate evenness and total substrate activity; Fig. 3). Moreover, all four indexes were positively correlated to degree of openness resulting from Hurricane Hugo. Differences related to hurricane disturbance were significant or approached significance at all inoculation times for all indexes, except for total substrate activity at 72 h incubation and substrate richness at 24 and 36 h. In contrast, the effects of previous landuse history had little detectable effect on any index of bacterial diversity after accounting for the effects of natural disturbance (Fig. 3). The only situation in which landuse approached significance in the ANCOVA occurred for substrate richness at 36 h. Even the more powerful *a priori* contrasts only detected a landuse effect that approached significance in 4 of 40 contrasts: total substrate activity at 24 and 36 h, and substrate richness at 36 and 48 h. None of the *a posteriori* comparisons of landuse categories even approached significance. The amount of variation in an index (R^2) explained by hurricane damage and landuse was relatively consistent regardless of inoculation time (*i.e.,* substrate diversity, 23.6–30.7%; substrate richness, 20.6–25.3%; substrate evenness, 23.9–25.3%; total substrate activity, 23.3–35.9%).

The apparent failure of landuse history to account for current bacterial diversity may be related to spatial correspondence between hurricane damage and previous landuse. The MANOVA assessing differences in degree of hurricane damage among landuse categories was significant (Wilks' Lambda = 0.729; F = 3.076; df = 4, 72; P = 0.021). Thus, the degree of hurricane damage was not independent of landuse history. Associations between landuse and topography as well as between landuse and tree species composition (*e.g.,* Fig. 2) likely affect the correlation between landuse and hurricane impact. Importantly, statistical removal of the effects of the hurricane may remove correlated effects due to landuse, thereby confounding interpretations of the latter. Indeed, ANOVAs assessing the effect of landuse without adjusting for the impact of Hurricance Hugo frequently detected landuse-specific differences in all four aspects of functional diversity (not shown).

These results demonstrate patterns in the spatial organization of functional diversity that are significantly related to spatial variability in the effects of Hurricane Hugo, regardless of landuse history and despite considerable microspatial variation. It is clear that hurricane impacts are not uniform across landscapes; landform and topography modify wind speed and influence patterns of vegetation damage (Bellingham 1991, Brokaw & Grear 1991). In the LEF, damage to trees generally was greater on north-facing slopes and on exposed ridges (Brokaw & Grear 1991, Walker 1991, You & Petty 1991). Moreover, tree species demonstrated differential succeptibilities to damage and recovery (Guzmán-Grajales & Walker 1991, Walker 1991). In fact, the patchy nature of hurricane damage led Brokaw and Grear (1991) to reiterate the hypothesis presented by Doyle (1981) that hurricane disturbance may increase the structural complexity of forest communities in the LEF. Clearly, hurricane-related changes in aboveground community structure are paralleled by differences in functional diversity among belowground communities.

Many mechanisms underlie changes in soil bacterial diversity as a result of hurricane damage to aboveground communities. Immediate impacts of Hurricane Hugo included abrupt changes in microclimate (Fernández & Fetcher 1991) resulting from defoliation and mortality of vegetation (Brokaw & Grear 1991, Walker 1991), as well as massive litter inputs (Lodge *et al.* 1991). Intermediate-term effects in the months following Hurricane Hugo included changes in plant community structure (Brokaw & Grear 1991, Walker 1991) and associated microclimate (Fernández & Fetcher 1991), root turnover (Parrotta & Lodge 1991), and soil nutrient dynamics (Lodge *et al.* 1991, Steudler *et al.* 1991). Long-term consequences likely include changes in plant community structure and composition, root-soil interfaces, soil organic matter content and nutrient dynamics (Sanford *et al.* 1991). Although specific causal relationships are uncertain, Vinton and Burke (1995) found that microbial biomass and N mineralization rates also varied in prairie ecosystems, according to the density and composition of the aboveground plant community. This suggests pervasive linkages between above- and belowground communities in terms of structure and function.

It is clear that microsite variation is high enough to account for the majority of variation within a landuse category, but does not overwhelm variation among landuse categories or variation related to the degree of hurricane damage. However, it is not clear how much variation is due to hurricane vs. landuse disturbance (which are related spatially). After all, landuse is influenced by topography, aspect, and soil characteristics, which also affect ecosystem structure (including soil features such as depth, chemical composition, SOM content, water availability, etc.). All of these factors tend to be correlated, so it is not clear, in the absence of manipulative experiments, which factors are most important in structuring soil communities.

GUILD-SPECIFIC ANALYSES.—In general, the effect of anthropogenic and natural disturbance was heterogeneous with regard to the six guilds, regardless of index. Moreover, the heterogeneity of response was not equivalent for all indexes (Table 2). For example, the effect of Hurricane Hugo on the catabolism of miscellaneous compounds at least approached significance for three of four indexes at most incubation times. The major exception to this pattern was for substrate richness, which showed no effect of the hurricane at any time. Index values based on carbohydrate use also revealed effects of the hurricane at least approaching significance at most incubation times, with substrate richness at 24 and 36 h being the only exceptions. In contrast, for amides and amines, the effect of Huricane Hugo was not detected at any time for substrate richness, substrate diversity, or substrate evenness, but at least approached significance at all times for

TABLE 2. *Results of analyses of covariance for indexes of functional diversity as a consequence of historical landuse and hurricane damage. A separate analysis was conducted for each guild at each inoculation time. Significance for the covariates assesses the consequences of hurricane damage whereas significance for the categorical factor, landuse, evaluates mean difference after the effect of the covariates has been removed. Codes for significance are @, $0.10 \geq P > 0.05$; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$. Time is hours of incubation.*

		Time				
Guild	Source	24	36	48	60	72
Substrate diversity						
Miscellaneous	Covariates	@	*	*	*	*
	Landuse	ns	ns	ns	ns	ns
Amines and amides	Covariates	ns	ns	ns	ns	ns
	Landuse	ns	**	ns	ns	ns
Carbohydrates	Covariates	*	*	**	**	*
	Landuse	ns	ns	ns	ns	ns
Carboxylic acids	Covariates	ns	@	ns	@	*
	Landuse	ns	ns	ns	ns	ns
Polymers	Covariates	*	@	ns	ns	ns
	Landuse	ns	ns	ns	ns	ns
Amino acids	Covariates	ns	ns	ns	ns	ns
	Landuse	ns	*	ns	ns	ns
Substrate richness						
Miscellaneous	Covariates	ns	ns	ns	ns	ns
	Landuse	ns	ns	ns	ns	ns
Amines and amides	Covariates	ns	ns	ns	ns	ns
	Landuse	ns	**	ns	ns	ns
Carbohydrates	Covariates	ns	ns	@	*	*
	Landuse	ns	ns	ns	ns	ns
Carboxylic acids	Covariates	ns	ns	@	ns	ns
	Landuse	ns	*	*	ns	@
Polymers	Covariates	ns	ns	ns	ns	ns
	Landuse	ns	ns	ns	ns	ns
Amino acids	Covariates	ns	ns	ns	ns	ns
	Landuse	ns	**	@	ns	ns
Substrate evenness						
Miscellaneous	Covariates	ns	*	*	@	ns
	Landuse	ns	ns	ns	ns	ns
Amines and amides	Covariates	ns	ns	ns	ns	ns
	Landuse	ns	ns	ns	ns	ns
Carbohydrates	Covariates	*	*	**	*	@
	Landuse	*	ns	ns	ns	ns
Carboxylic acids	Covariates	ns	ns	ns	ns	ns
	Landuse	ns	ns	ns	ns	ns
Polymers	Covariates	@	*	*	ns	ns
	Landuse	ns	*	ns	ns	ns
Amino acids	Covariates	ns	ns	ns	@	ns
	Landuse	ns	ns	ns	ns	ns
Total activity						
Miscellaneous	Covariates	@	*	*	*	@
	Landuse	ns	ns	ns	ns	ns
Amines and amides	Covariates	@	@	*	@	@
	Landuse	ns	ns	ns	ns	ns
Carbohydrates	Covariates	*	**	*	*	@
	Landuse	ns	ns	ns	ns	ns
Carboxylic acids	Covariates	ns	@	ns	ns	ns
	Landuse	ns	ns	ns	ns	ns
Polymers	Covariates	ns	ns	ns	ns	@
	Landuse	ns	ns	ns	*	*
Amino acids	Covariates	ns	*	@	ns	ns
	Landuse	ns	ns	ns	ns	ns

total substrate activity. Patterns of hurricane-related effects on the functional diversity of other substrates varied, but in all cases, index values were positively correlated to canopy openness.

Differences in diversity indexes among categories of landuse, after effects of the hurricane were removed, were rarely found to be significant. Indexes based on the catabolism of miscellaneous compounds were never significantly related to landuse, and for carbohydrates, only substrate evenness at 24 h incubation showed a significant effect. Diversity indexes based on use of carboxylic acids, amines and amides, and amino acids showed a significant landuse effect in two of twenty tests per guild. For amines and amides, and amino acids, landuse had a significant effect on sustrate diversity and substrate richness at 36 h. In all, effects of landuse on functional diversity at least approached significance in only 12 of 120 tests, with no consistent pattern appearing for any particular index. However, these results should be interpreted with caution because the effects of landuse and hurricane are confounded spatially on the HRP.

CONCLUSIONS

We have demonstrated that despite considerable microspatial variation, prior disturbance of the landscape resulting from natural and anthropogenic stresses has a strong impact on contemporary spatial organization of the functional diversity of soil bacterial communities. Moreover, natural and anthropogenic disturbances may not be independent, to the extent that topography and prior disturbances affect the subsequent susceptibility of sites to additional disturbance-mediated processes. Indeed, failure to account for the heterogeneity of human landuse practices culminating 50 yr ago, as well as more recent hurricane-induced variability (5 yr ago) on the HRP would lead to spurious or misleading conclusions concerning the spatial organization of functional diversity. The legacy of disturbance will become an increasingly important consideration at all levels of ecological organization as natural systems become increasingly affected by human activities. Nonetheless, our results document that functional diversity of soil bacterial communities increases with degree of disturbance, at least within the context and range of disturbances measured on the HRP.

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