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# Characterization of soil ecosystems in Costa Rica using microbial community metrics

W. D. EATON[1] & O. CHASSOT[2]

*[1]School of Environmental and Life Sciences, Kean University, Union, New Jersey 07083, USA*
*[2]Centro Científico Tropical (CCT), Apdo 8-3870-1000 San José, Costa Rica*

**Abstract:** The goals of the research presented in this issue of *Tropical Ecology* were to demonstrate that differences in soil DNA-based microbial community characteristics, biomass, microbial activity, and C and N nutrient dynamics can be used as indicators of environmental change, specifically to show how land management, restoration strategies and climate change may be impacting the soil ecosystems of lowland forests and montane cloud forests of Costa Rica. In the studies conducted in lowland secondary forests, the bacterium *Frankia* was found to be critical for nitrogen-fixation in the soils associated with nitrogen-fixing tree *Pentaclethra macroloba,* but *Frankia, Rhizobium,* Archaea, and Type II methanotrophs were also collectively important in recuperation of the soil N and in enhancing microbial biomass C via more efficient use of organic C. These same secondary forest soils had greater levels of inorganic P and N, organic carbon, and microbial activity, and were more fungal-dominant soil ecosystem than grasslands of the same age. In an adjacent primary forest, the bromeliad *Bromelia pinguin* was found to demonstrate significant antifungal effects on the forest soil, resulting in decreases in fungal abundance, microbial biomass, and efficiency of organic C use. Lastly, in the Monteverde cloud forests, microbial metrics were used to show that habitats with the greatest soil moisture had more fungal-dominated soils, and unique fungal and bacterial population compositions. Given the importance of tropical soils in global C storage, their degradation, and current restoration efforts in these forests, as well as the very clear threat of decreases in precipitation due to climate change, belowground metrics should be considered as early indicators of the effects of forest restoration and/or further environmental disturbances. We hope that these articles will stimulate future studies in these important areas of Costa Rica.

**Resumen:** Las metas de la investigación presentada en este número de *Tropical Ecology* fueron demostrar que las diferencias en las características de la comunidad microbiana del suelo basadas en ADN, la biomasa, la actividad microbiana y las dinámicas del C y del N de los nutrientes pueden ser usadas como indicadores de cambio ambiental, específicamente para mostrar cómo el manejo de la tierra, las estrategias de restauración y el cambio climático pueden tener impactos sobre los ecosistemas edáficos de los bosques de tierras bajas y los bosques nublados montanos de Costa Rica. En los estudios realizados en bosque secundarios de tierras bajas, se encontró que la bacteria *Frankia* tiene un papel crítico en la fijación de nitrógeno en los suelos asociados con el árbol fijador de nitrógeno *Pentaclethra macroloba*, pero además que *Frankia, Rhizobium*, Archaea y los metanotrofos Tipo II también fueron importantes de manera conjunta en la recuperación del N del suelo y en el aumento del C en la biomasa microbiana gracias a un uso más eficiente de C orgánico. Estos mismos suelos de los bosques secundarios tuvieron niveles más altos de P y N inorgánicos, carbono orgánico y actividad microbiana, y constituyeron un ecosistema edáfico con mayor dominancia de hongos que los pastizales de la misma edad. En un bosque primario contiguo se demostró que la

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* *Corresponding Author*; e-mail: weaton@kean.edu

bromeliácea *Bromelia pinguin* tiene efectos antifúngicos significativos en el suelo forestal, lo que provoca una reducción en la abundancia de hongos, la biomasa microbiana y la eficiencia en el uso del C orgánico. Finalmente, en los bosques nublados de Monteverde se usaron métricas microbianas para mostrar que los hábitats que tenían la mayor humedad del suelo tenían suelos con mayor dominancia de hongos, así como composiciones únicas de las poblaciones de hongos y bacterias. Dada la importancia de los suelos tropicales en el almacenamiento mundial de C, su degradación y los esfuerzos actuales de restauración en estos bosques, así como la amenaza muy clara de decremento en la precipitación debido al cambio climático, las métricas del subsuelo deberían ser consideradas como indicadores tempranos de los efectos de la restauración forestal y/o de otros disturbios ambientales. Esperamos que estos artículos estimulen el desarrollo de futuros estudios en estas áreas importantes de Costa Rica.

**Resumo:** Os objectivos da investigação apresentada nesta edição da *"Tropical Ecology"* foram o de demonstrar que as diferenças do DNA do solo, baseadas nas características da comunidade microbiana, biomassa, atividade microbiana e dinâmicas dos nutrientes do C e N podem ser usadas como indicadores de alterações ambientais, especialmente para mostrar como a gestão do solo, as estratégias de restauração e mudanças climáticas podem impactar nos ecossistemas do solo das florestas das várzeas e das florestas montanas de nuvens da Costa Rica. Nos estudos conduzidos nas florestas secundárias de várzea encontrou-se que a bactéria *Frankia* era crítica para a fixação do N no solo, associada a árvore fixadora de azoto, a *Pentaclethra macroloba.* Refere-se que a *Frankia*, o *Rhizobium*, Archaea, e metanotrofos de Tipo II eram, também, colectivamente importantes na recuperação do N do solo e na intensificação do C da biomassa microbiana por via do uso mais eficiente do C orgânico. Estes mesmos solos de florestas secundárias apresentavam maiores níveis de P e N inorgânicos, carbono orgânico e atividade microbiana, e eram solos com ecossistemas mais dominados pelos fungos do que as pastagens da mesma idade. Na floresta primária adjacente, a bromelídia, *Bromelia pinguin*, pareceu demonstrar efeitos antifúngicos significativos no solo florestal de que resultava um decréscimo na abundância da biomassa fúngica e na eficiência no uso do C orgânico. Finalmente, na floresta de nuvens de Monteverde, usaram-se métricas microbianas para mostrar que os habitats com a maior humidade no solo têm mais solos dominados pelos fungos e composições das populações fúngicas e bacterianas únicas. Dada a importância dos solos tropicais no armazenamento global de C, a sua degradação, e os esforços atuais de restauração destas florestas, assim como as claras ameaças de decréscimo na precipitação devido à mudança climática, as métricas do subsolo devem ser consideradas como indicadores precoces dos efeitos da restauração florestal e/ou distúrbios ambientais futuros. Esperamos que este artigo sirva de estímulo a estudos futuros nestas importantes áreas da Costa Rica.

**Key words:** Climate, land management, microbial communities, soil ecosystems, tropical forests.

## Soil microbes as indicators of habitat change

The quantity and composition of different key microbial groups drives the nutrient processes in soils (for examples see Bardget *et al.* 2005; Chen & Xu 2010; Kardol & Wardle 2010; Knief *et al.* 2005; Knorr *et al.* 2005; Leininger *et al.* 2006; Nielsen *et al.* 2011; Talbot *et al.* 2008; Wardle 2006). Fungi generally decompose organic substrates more efficiently than bacteria, leaving behind more organic residues to enhance the organic carbon content in soil. The saprotrophic fungi are generally thought of as producers of extracellular enzymes (such as laccases, proteases, and chitinases) and serve as the primary agents of degradation of the more complex organic molecules, fulvic and humic substances, and are critical in decomposition in soil and the litter layer. The soil bacteria are critical in N and nutrient cycling and decomposition in managed soils, younger soils, or soils recovering from disturbance. The saprotrophic bacteria are important for the degradation of simpler organic compounds (such as organic acids, carbohydrates, amino acids, etc.) in soil. The nitrogen-fixing bacteria function to convert dinitrogen into ammonium, and the nitrifying bacteria oxidize the ammonium into nitrate. The methylotrophs are important for oxidizing methane produced, and are good indicators of habitat condition, as their activity and abundance decreases with soil damage. The role of Archaea in forest soils is not yet clear, but they appear to be important in soil C and N nutrient processes, and warrant more attention in forest ecology studies.

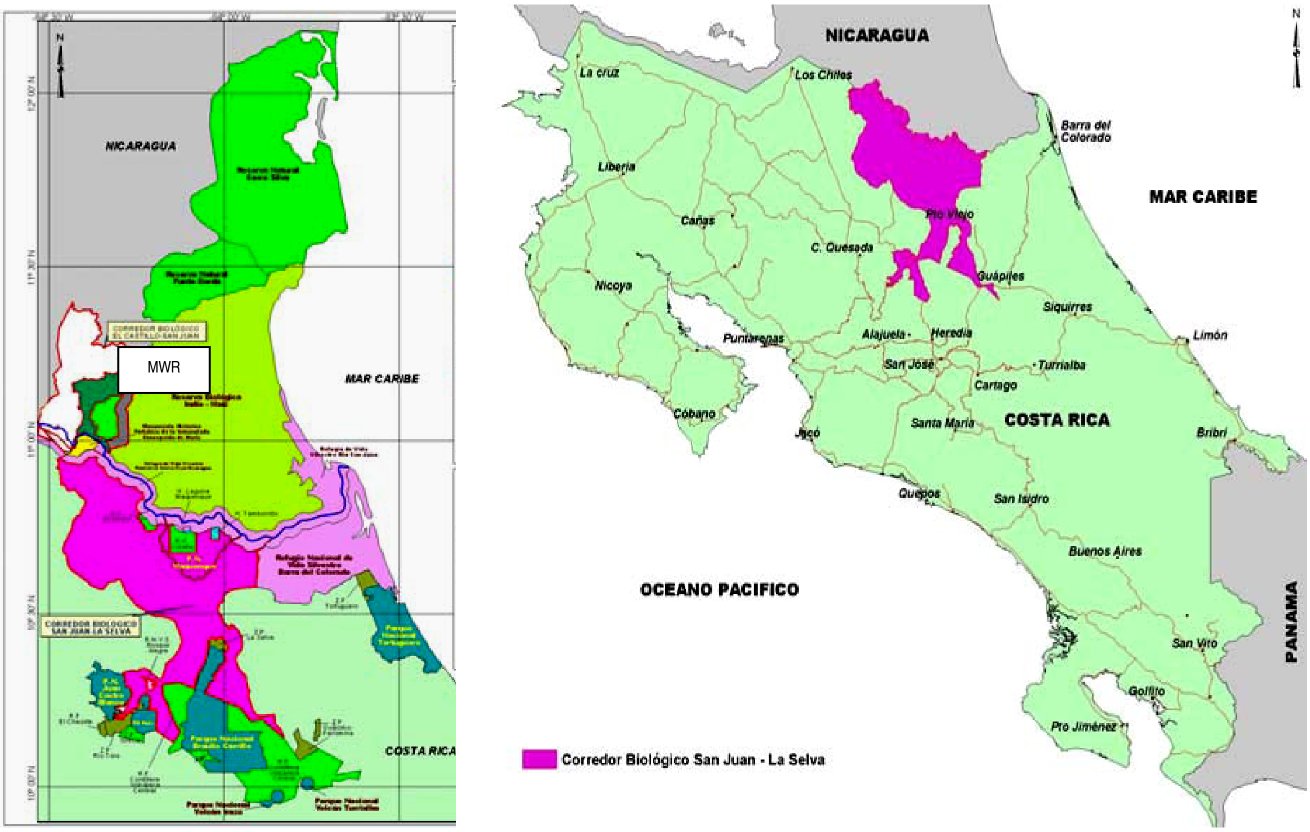
As the composition of the soil microbial communities rapidly responds to variations in environmental conditions that impact ecosystem functioning, changes in these communities are good indicators of environmental impact. For example, land management practices and changes in climate have been associated with decreases in bacterial-based N-cycle activities, resulting in decreased microbial biomass and increased concentrations of pools of inorganic N (Ewing *et al.* 2007) and lower concentrations of P (Campo *et al.* 2001; McGrath *et al.* 2000). These conditions inhibit N-fixation (King & Purcell 2005; Pons *et al.* 2007; Reed *et al.* 2007; Schulze 2004), suppress fungal biomass development (Bittman *et al.* 2005; de Vries *et al.* 2007) and the decomposition of lignin and lignaceous material (Knorr *et al.* 2005; Sinsabaugh *et al.* 2002, 2004; Waldrop & Zak 2006). Such changes in environmental conditions and nutrient components have been associated with changes in the composition of basidiomycete and arbuscular mycorrhizal fungi, N-fixing bacteria, ammonium oxidizing bacteria, and saprotrophic bacteria communities (Andrews & Harris 1986; Axelrood *et al.* 2002; Eaton *et al.* 2011; Fierer *et al.* 2007; Griffith & Bardgett 2000; He´ry *et al.* 2005; Holland & Coleman 1987; McCaig *et al.* 1999; Padmanabhan *et al.* 2003).

## Tropical lowland rainforests

The lowland rainforest ecosystems in Costa Rica are distinguished by a high density of Almendro tree (*Dipteryx panamensis*), which has become the primary source of hard wood for flooring, truck beds, and other specialized uses (Chassot *et al.* 2005). Moreover, as this tree species has become scarcer, the price for the wood has increased dramatically, providing even greater incentives to cut the trees, resulting in inconsistent land management practices that have caused disturbances to many of the ecosystems in which these trees are found. The lowland forests of the Northern Zone of Costa Rica have been the most impacted by this practice, resulting in the highest deforestation rate in the country over the last two decades, with less than 30 % of the original forest standing (Monge *et al.* 2002).

These practices have resulted in significant fragmentation of the lowland rainforest ecosystems in which mosaics of smaller tracts of fragmented primary forest or selectively harvested forest are surrounded by larger tracts of degraded lands. Regional scientists and natural resource managers in Costa Rica are concerned that, because the area of primary lowland rainforests are small and diminishing, it is unclear whether the remaining primary forests will be able to regenerate at a rate sufficient to match the deforestation (Chassot *et al.* 2001). Another concern is that, as the forest is converted for other uses, not only is the acute loss of forested land felt, but that the “edge effect” will continue to degrade adjacent remaining forest biomass (Laurance *et al.* 1997).

The San Juan-La Selva Biological Corridor (Fig. 1) was established in 2001, with the support of the Costa Rican Ministry of Environment and Energy (http://www.lapaverde.or.cr/ index_eng.htm). The corridor links six protected areas in the region into a single integrated biological unit totaling 1,204,812 ha. The core conservation unit of the San Juan-La Selva Biological Corridor is the new Maquenque National Wildlife Refuge (MNWLR; Fig. 1), situated immediately south of Indio Maíz and abutting the western boundary of Barra del Colorado. This new refuge conserves the portion of the corridor with the highest percentage of forest cover. The size and location of the MNWLR and surrounding biological corridor is based on scientific data identifying the most valuable habitat for biodiversity (Chassot & Monge 2006). The area is composed of a variety of habitat types, including old-growth or primary forests, selectively logged forests, secondary forests that are up to about 25 years old, open pasture used for grazing, monoculture tree plantations and other agricultural plantation types, and areas that include land which has been degraded to the point of being considered near useless for production or restoration. The SJLSBC Executive Committee developed a *Research and Monitoring Plan* to, in part, deve-



Maquenque National Wildlife Refuge (MNR)

Costa Rica and the San Juan La Selva Biological Corridor

**Fig. 1**. A map of Costa Rica, including the San Juan-La Selva Biological Corridor protected area, with the Maquenque National Wildlife Refuge (MNR) marked in purple, and a map of the MWR. (Maps from the San Juan La Selva Biological Corridor Commission, www.lapaverde.or.cr).

lop an initial snapshot of multiple trophic levels of ecosystem activity to identify overall ecosystem health indicators; develop and test a suite of tools for assessing ecosystem health in the area; and use these to assess both positive and negative impacts of current and former land management strategies.

During the summer of 2009, we conducted studies in the MNWLR to begin to characterize the soil biotic nutrient composition associated with the soil ecosystems within the forest and grassland habitats. The focus of the first study presented in this issue of *Tropical Ecology* (Lowe *et al.* 2012) concerning *Penetaclethra macroloba* was to determine how the tree might impact soil rhizobial, archaea, and methanotroph communities and the C and N nutrient dynamics within secondary forests. The second study presented (Eaton *et al.* 2012a) was the first attempt at identifying how *Penetaclethra macroloba* influences the N-fixing microbial community within the soils. These projects are of relevance as *Pentaclethra macroloba* is the dominant tree with nitrogen (N)-fixing root nodules in these lowland forests of Costa Rica (Hartshorn & Hammel 1994; Pons *et al.* 2007), and as such is presumed to be an important early colonizing tree in secondary forests in this region. There is also some discussion about the possible use of these trees as part of restoration strategies to recuperate cleared forest lands (Nelson Zamora, INBio, personal communication). However, little is known about the N cycle dynamics and N-fixing bacteria associated with this tree. The study presented by Hafich *et al.* (2012) was designed to compare the soil biotic and nutrient characteristics in secondary forests and adjacent grasslands. The purpose how the soil ecosystems were affected by conversion of primary forest to grassland and to secondary forests. Lastly, a project by Looby *et al.* (2012), describes for the first time the affects that the commonly occurring terrestrial bromeliad, *Bromelia pinguin*, has on soil fungal and bacterial populations, C and N nutrients. Of particular note is the apparent antifungal properties this plant seems to have on soil fungi.

### *Role of Pentaclethra macroloba in N-cycling and N-Recuperation within secondary forests*

Lowe *et al.* (2012) studied the C and N nutrient dynamics and the diversity and complexity of the *Rhizobium*, Archaea, and Methanotroph communities in the *Pentaclethra macroloba*-dominant and adjacent secondary forest soils. They found that the soil from the more complex vegetation community of the secondary forests had greater microbial biomass, and demonstrated greater efficiency of C use than the *Pentaclethra*-dominant soils-typical of a more advanced soil. *Rhizobium* was also more abundant in the *Pentaclethra*-dominant soils, and Archaea was more abundant in the secondary forest soils, whereas both soil types had similar levels of inorganic N. The greater abundance of Archaea associated with greater biomass and efficiency of organic C use suggests that they may be playing an important role in rhizosphere C soils, as has been suggested by others (Chelius & Triplett 2001; Simon *et al.* 2000, 2005; Sliwinski & Goodman 2004). Quaiser *et al.* (2002), Pernthaler *et al.* (2008), and Miyazaki *et al.* (2009) have all identified nifH gene-like clusters in Archaea, providing some of the first evidence that these microbes may be involved in N-fixation. This could account for the patterns observed in this study, however, this needs to be confirmed. Nonetheless, this work shows that there are ecologically important interactions and community structural changes occurring among the *Rhizobium* and Archaea populations within these soils that affect C and N cycling.

Eaton *et al.* (2012a) found that the *Pentaclethra* root nodules were likely influencing the N-fixing bacterial communities in the non-rhizosphere soils of these *Pentaclethra*-dominant forests, and in the non-rhizosphere soils immediately adjacent to the trees. It appears that *Frankia* is much more critical than *Rhizobium* in recuperating N in the non-rhizosphere forest soils, as the N component levels were the same, but the abundance of *Frankia* rRNA was much greater than *Rhizobium* rRNA in both soil types. The data also suggest that the non-rhizosphere soils immediately adjacent to the trees are benefitting from the *Pentaclethra* root nodule community generating a greater microbial biomass and more efficient use of C than the rest of the non-rhizosphere soils in the forest.

The results of these two studies begin to show the complexity of the N-fixing soil community associated with *Pentaclethra macroloba* and the adjacent secondary forests in the tropical lowlands. It is clear that there is a complex community of microorganisms probably associated with N-fixation in these soils that includes *Frankia, Rhizobium,* Archaea, and Type II Methanotrophs (and probably others), which collectively help to recuperate the soil N and enhance microbial biomass C via more efficient use of organic C. These studies provide support for the use of *Pentaclethra macroloba* as part of forest restoration strategies in these Northern Zone forests of Costa Rica.

### *Comparisons of soil ecosystem conditions within secondary forests and adjacent grasslands*

Tropical soils contain about 20 % of the global C storage (Jobbágy & Jackson 2000), but have suffered significant deforestation, with concomitant ecosystem damage, increased release of greenhouse gases, and decreases in C sequestration. However, land management strategies have been moving towards reforestation and the development of secondary forests as an important restoration strategy (Guo & Gifford 2002; Post & Kwon 2000; Wright 2005). Chazdon *et al.* (2007) discussed the rates of changes in vegetation occurring in these forests following the original disturbance leading to the development of productive forests, however, little is known of the composition and function of the microbial community in these secondary soils. Several studies reported in this issue of *Tropical Ecology* were conducted to begin analyzing the C and N nutrient dynamics, microbial activity and biomass, and the abundance, and diversity of the bacteria and fungi in the secondary forests and adjacent grasslands within the MNWLR. The purpose was to help understand the relationships between microbial community diversity and structure, nutrients, and the microbial biomass component of soil C storage in tropical secondary forests, and how these are affected by the use of secondary forests vs. grasslands as management strategies.

In these tropical secondary forest and grassland soils in the MNWLR, Hafich *et al.* (2012) compared the abundance and diversity of the N-fixing bacteria *Rhizobium* with the critical functional gene nifH, and the abundance and diversity of fungal rRNA with the key functional gene for the lignin degradation enzyme laccase, along with C use efficiency, and phosphate levels, as well as other C and N indicators. The data showed that the secondary forest had greater levels of phosphate, inorganic nitrogen, organic carbon, respiratory activity, abundance and diversity of Basidomycete rDNA, abundance of fungal rDNA, and lower abundance - but greater diversity - of *Rhizobium* rDNA, as well as lower abundance of nifH gene DNA than soils from adjacent grasslands of the same age. There were also significant correlations found between the abundance of Basidiomycete rDNA and of laccase gene with the levels of phosphate, microbial biomass, organic carbon use efficiency, and soil moisture content. These data are consistent with what has been reported by others in studies of tropical forests that have been converted to pasture in that a decrease in P availability results in a decrease in microbial biomass and activity, and C use efficiency (Allison *et al.* 2007; Cleveland & Townsend 2006; Cleveland *et al.* 2004; Cruz *et al.* 2009). The greater abundance of genetic indicators of N-fixation, along with lower levels of inorganic N in the grassland soils, suggests that (i) the secondary forest *Rhizobium* community may more active N-fixers; (ii) that there are other N-fixers in the secondary forests - such as Archaea and/or Type II Methanotrophs; and/or (iii) there is less ammonium oxidizing activity occurring. These data provided more specific evidence of the overall microbial activity, the interactions between microbes and P, the microbial activity, and the efficiency of C utilization as possible indicators of a more ecologically complex or advanced soil ecosystem resulting from the development of secondary forests.

### *Role of Bromelia penguin on nutrient cycle dynamics and microbial populations in a tropical primary forest*

The primary forests within the MNWLR are typical of the region, the tree community composition and structure having been previously characterized (McNulty & Barry 2009; Quirós-Brenes 2002). However, little is known about the ground cover vegetation composition, structure and function in these forests. *Bromelia pinguin* (Bromeliaceae) is a conspicuously common plant in the primary forests of the MNWLR and throughout Central America and the Caribbean (Hallwachs 1983; Woodley & Janzen 1995). Known for its spiny fronds, this bromeliad deters grazers and large mammals, and provides a sanctuary for many small insects and invertebrates. The ecology of this bromeliad is not well understood. The fruit pulp is very acidic (Payrol *et al.* 2005), it is used as an antihelminthic and to treat whooping cough and scurvy (Camacho-Hernández *et al.* 2002), and it is a great source of natural proteases, especially pinguinain (Toro-Goyco *et al.* 1968), which Camacho-Hernández *et al.* (2002) showed had significant antifungal activity.

Looby *et al.* (2012) conducted a study to determine how this bromeliad affected the below-ground nutrient cycling processes and microbial communities in a primary forest, zones of bromeliads within that primary forest, and in the ecotone areas between the bromeliad zone and the primary forest within the MNWLR. The goal was to determine how this plant affected microbial biomass and organic content, fungal populations and associated C-cycle dynamics, and Rhizobial populations and N-cycle dynamics. The data showed that *Bromelia pinguin* may be inhibiting fungi and basidiomycetes in these soils in comparison to the adjacent forest soils, reducing the capacity to decompose organic C, and altering the nutrient cycle components. The abundance and diversity of rhizobial, fungal, and basidiomycete DNA, the microbial activity, microbial biomass, efficiency of organic C use, and the levels of P and $NH_4$ were all greater in the primary forest and ecotone soils than in the bromeliad soils suggesting more complex and fungal-dominated soil ecosystems in the primary forest and ecotone area than in the bromeliad patches. There was a decrease in the complexity but not the functionality of the rhizobial community in the bromeliad soils, as the amount of N fixation did not change, but the species composition (?) of the N-fixing community did.

## Tropical montane cloud forests

Tropical montane cloud forests support about 16 % of the planet's vertebrate and 20 % of the plant diversity, and require both rain and frequent immersion in clouds for their maintenance. Immersion in the cloud layer provides 15 - 100 % of the moisture available during the dry season (Bruijnzeel & Proctor 1993). Many of the tropical montane cloud forests around the world are already severely threatened (Foster 2001; Nadkarni & Wheelwright 2000), including those of Monteverde, Costa Rica (Lawton *et al.* 2001, 2010; Nair *et al.* 2003; Pounds *et al.* 2004, 2006; Ray *et al.* 2006). Monteverde is a large biological reserve that straddles the Pacific and Atlantic sides of the continental divide. It is completely surrounded by a combination of cleared land that was formerly forested, and has now been allowed to regenerate to secondary forest and grasslands.

Studies in Monteverde have provided cloud and climate models suggesting that altitude of the cloud base layer over these forests may be increasing, reducing the amount of moisture available, especially in the dry season, and altering the characteristics of the ecological communities within these unique forest types (Lawton *et al.* 2001, 2010). Causes for the increased altitude of the cloud layer and the decrease in cloud-covered surface area are thought to include both increases in sea surface temperatures and atmospheric $CO_2$ (Pounds *et al.* 1999) as well as lowland deforestation (Lawton *et al.* 2001, 2010; Nair *et al.* 2003). Population declines of anurans, migration of birds to higher elevations, changes in plant communities, and the extinction of the harlequin frog (*Atelopus varius*), and golden toad (*Bufo periglenes)* have been observed recently in the Monteverde cloud forest, and have been attributed by some to the increasing cloud base heights (Colwell *et al.* 2008; Foster 2001; Pounds & Puschendorf 2004; Pounds *et al.* 1999, 2006; Thomas *et al.* 2004).

As a result of these issues, Olivier Chassot (World Commission on Protected Areas and Universidad para la Cooperación Internacional, in Costa Rica) established a new consortium of scientists to characterize the ecosystem parameters in 6 different habitats within different Holdridge life zones in the Monteverde Reserve. The goals of this project are to characterize the ecological conditions, communities, and diversity of the birds, reptiles, insects, vegetation, soil chemistry, and microbes in these habitats, and establish a monitoring program to assess the different trophic level structures for change over time associated with climate change (Salazar *et al.* 2009). The study by Eaton *et al.* (2012b) is the first to identify metrics for use in monitoring soil ecosystem health in these habitats over time, and to begin establishing a baseline of soil community characteristics.

Eaton *et al.* (2012b) compared the soil moisture, N-fixation activity, microbial biomass, fungal and bacterial abundance and diversity, and the abundance of the key functional genes laccase (for lignin degradation by basidiomycete fungi) and nifH (for bacterial N-fixation) among the habitats representing the different Holdridge Life Zones. They showed that the habitats with the greatest soil moisture on both the Pacific and Caribbean slopes had the greatest amount of fungal abundance, suggesting a more fungal-dominated soils, and also had clearly unique fungal and bacterial populations. The more moist habitats from both slopes seemed to have more active N- fixation and incorporation of mineral N into the biomass, and appeared to select for N-fixing bacteria and basidiomycete fungi, as well as lignin degradation. The authors considered the metrics that most influenced or were influenced by soil moisture and microbial biomass as target indicators of "soil quality". The abundance of fungal rRNA, fungal-to-bacterial rRNA ratio, and abundance of laccase and nifH genes were positively correlated with soil moisture and microbial biomass, whereas total mineral N levels and abundance of bacterial rRNA were negatively correlated with soil moisture and microbial biomass.

This study demonstrated that biologically meaningful differences – representing important ecological relationships – do exist in the soil nutrients, microbial community, and microbial biomass in the different habitats in Monteverde that were correlated with soil moisture. These data suggest that these microbial community indicators can be used to help describe and understand important soil ecosystem relationships, and can be used for monitoring changes in soil ecosystem conditions should moisture levels decrease in the Monteverde forests.

## Summary

The studies presented in this issue of *Tropical Ecology* show that microbial community characteristics, biomass, microbial activity, and C and N nutrient dynamics can be used to demonstrate differences within disturbed and restored habitats, and in habitats experiencing differences in soil moisture, and could be used as indicators of environmental change. Anderson (2003), Doran (2002), and Wardle *et al.* (2004), among others, have argued that, because the belowground parameters change rapidly with environmental disturbances, and greatly affect the aboveground vegetation, they should be used as ecosystem indicators in monitoring programs. We suggest that, given the importance of the tropical soils in global C storage, the deforestation and land degradation that has occurred in the tropics, as well as current restoration efforts in the lowland tropical forests, and the very clear threat of decreases in moisture in the montane cloud forests, we need to start using these belowground metrics now to elucidate ecological relationships in these soils, and to monitor them for effects of restoration as well as future disturbances.

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