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Enzyme activities and metabolic profiles of soil microorganisms at KILN sites in *Quercus* spp. temperate forests of central Mexico

Blanca Estela Gómez-Luna[a,*], Graciela Ma de la Luz Ruiz-Aguilar[b], Gerardo Vázquez-Marrufo[c], Luc Dendooven[d], Víctor Olalde-Portugal[e]

[a] *Departamento de Ingeniería Agroindustrial, División de Ciencias de la Salud e Ingenierías, Campus Celaya-Salvatierra, Universidad de Guanajuato, Privada de Arteaga s/n Zona Centro, C.P. 38900, Salvatierra, Guanajuato, Mexico*

[b] *Departamento de Ciencias Ambientales, División de Ciencias de la Vida, Campus Irapuato-Salamanca, Universidad de Guanajuato, Ex Hacienda El Copal Km 9, Carretera Irapuato-Silao, C.P. 36500, Irapuato, Guanajuato, Mexico*

[c] *Centro Multidisciplinario de Estudios en Biotecnología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana de San Nicolás de Hidalgo, Km 9.5 Carretera Morelia-Zinapécuaro La Palma, Tarímbaro, C.P. 58262, Morelia, Michoacán Mexico*

[d] *Laboratorio de Ecología de Suelos, Cinvestav, Av. Instituto Politécnico Nacional 2508, San Pedro Zacatenco, 07360 Ciudad de México, Mexico*

[e] *Laboratorio de Bioquímica Ecológica, Departamento de Biotecnología y Bioquímica, Cinvestav Unidad Irapuato, Irapuato, Mexico*

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ABSTRACT

Temperate forest dominated by *Quercus* L. spp. cover large parts of central Mexico and rural communities depend on these forests for wood and charcoal. The impact of charcoal production on enzymatic and metabolic profiles of soil microorganisms in the 0–15 cm layer were investigated during the dry and rainy season. A KILN site, used for charcoal production, is prepared by clearing all vegetation, removing litter and soil from the surrounding area. The wood is piled up in the centre of the KILN site and covered with litter and soil. Incomplete combustion with a minimum of flame production is maintained for 12 days. The charcoal production had a negative effect on the functional diversity and enzymatic microbial activity in soil. Community level physiological profiles (CLPP) analyses showed a lower average well color development, substrate richness, and functional diversity in soil at the KILN sites compared to the undisturbed soil. Cluster analysis dendrograms and canonical discriminant analysis of CLPP indicated that substrate utilization at the KILN sites was different from undisturbed forest soil. The activity of six enzymes, i.e. CM-cellulase, β-glucosidase, N-acetyl-β-D-glucosaminidase, nitrate reductase, urease and proteinase, decreased from 44% to 90% at the KILN sites compared to the undisturbed forest soil. It was found that charcoal production at a KILN site showed lower functional diversity and enzymatic microbial activity than in the surrounding forest soil as a result of loss of litter and organic matter and changes in microclimatic conditions.

1. Introduction

Charcoal is used as fuel in rural communities in many places around the world (Manlay et al., 2000; Coomes and Burt, 2001; Glaser et al., 2002; Vázquez-Marrufo et al., 2003; Nepstad et al., 2006). Charcoal is made by burning wood in a KILN, creating internal suppressed combustion with the least possible flame (Vázquez-Marrufo et al., 2003). During the production lasting 12–14 days, a proportion of the biomass is transformed to charcoal (Pietikäinen and Fritze, 1993; Zackrisson et al., 1996). In the central highlands of Mexico charcoal is still produced in the Santa Rosa forest (Guanajuato). Typical KILN sites have a diameter of 7–10 m and up to 20 can be found per ha. As such, up to 3% of the forest can be covered by KILN sites. Ex-KILN sites have different climatic conditions, such as higher solar radiation, exposure to wind and water erosion, extreme temperatures and a greater UV light incidence, than in the surrounding forest (Vázquez-Marrufo et al., 2003).

The soil at ex-KILN sites is severely affected (Serrasolsas and Khanna, 1995; Prieto-Fernández et al., 1998). In a previous study in the Santa Rosa forest, the concentrations of exchangeable Ca^{2+}, K^{+} and Mg^{2+} increased >1.6 times at KILN sites and the pH increased from 4.5 in undisturbed soil to 7.0 at the KILN sites (Gómez-Luna et al., 2009). Soil organic matter content decreases at KILN sites decreasing soil microbial biomass C and N (Guinto et al., 1999; Oguntunde et al., 2004; Boerner et al., 2005; Guerrero et al., 2005). In the Santa Rosa forest the soil organic C at the KILN site was <50% of that found in the undisturbed forest and the microbial biomass C decreased 1.3 times in the rainy season and >2 times in the dry season. The microbial biomass composition changes, and

* Corresponding author. Tel.: +52 466 6632132; fax: +52 466 6633413.
E-mail address: bgomezl2000@yahoo.com.mx (B.E. Gómez-Luna).

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the number of bacteria and the basal respiration are reduced at KILN sites (Zackrisson et al., 1996; Prieto-Fernández et al., 1998; Pietikäinen et al., 2000; Glaser et al., 2002). In the Santa Rosa forest, the ammonifiers, nitrifiers and denitrifiers were 16 times lower at the KILN sites than in the undisturbed forest soil (Gómez-Luna et al., 2009).

Charcoal production has not only an immediate, but also a long-term effect on the environment (Zackrisson et al., 1996; Pitman, 2006). All vegetation is destroyed and reforestation can take years, erosion sets in further retarding the restoration of the ecosystem. Even 15 years after the production of charcoal, the soil has not recovered yet (Vázquez-Marrufo et al., 2003). Sustainable management of ecosystems requires an understanding of the factors that affect soil microbial populations, as there is a clear relationship between microbial diversity, soil quality and plant community (Mabuhay et al., 2003; Hart et al., 2005; Vásquez-Murrieta et al., 2006).

The functional diversity of heterotrophic microbial communities in soil has been examined using the BIOLOG® system (Garland, 1996a,b, 1997). This method determines the structure and metabolic capacity of microbial communities to use sole carbon sources. This provides a potential tool to monitor changes in microbial functional diversity in soil subjected to some kind of disturbance (Campbell et al., 1997; Staddon et al., 1997; Classen et al., 2003; Calbrix et al., 2005). The metabolic profiling technique is used for qualitative and quantitative characterization of microbial communities and it allows the use of multivariate statistical techniques to calculate and analyze diversity indices (Garland, 1997; Konopka et al., 1998; Bending et al., 2002).

The specific capacities of microbial communities in natural systems may be due to the presence of rare species, i.e. those that are either found in low density or those that are spatially dispersed (Zak et al., 1994). In this work multivariable analysis was used to investigate samples of the same habitat, but differentiated by charcoal production. Multivariable analysis has been used to compare microbial populations in different habitats, e.g. soil types (Garland and Mills, 1991; Zak et al., 1994).

Microplates have been used to determine the potential metabolic diversity of soil microbial community in sites affected by fire and high temperatures (Staddon et al., 1997; Staddon et al., 1998; Pietikäinen et al., 2000), as well as sites contaminated with heavy metals (Viti and Giovannetti, 2005), herbicides and pesticides (Ratcliff et al., 2006; Chen et al., 2007), hydrocarbons (Bundy et al., 2004) and changes in management or soil use (Yao et al., 2006; Govaerts et al., 2007).

Enzyme activity in soil is also used as an indicator of changes in quality and productivity of soil (Dick, 1994; Brookes, 1995; Eivazi and Bayan, 1996). It is applied to study short and long-term effects of a disturbance, as the biochemical reactions in soil are mediated by microorganisms (Burns, 1982; Eivazi and Bayan, 1996). These reactions are catalyzed by enzymes and play an important role in biogeochemical cycles (Eivazi and Bayan, 1996; Wittmann et al., 2004). As part of a study into the effects of charcoal production on soil functioning (Vázquez-Marrufo et al., 2003; Gómez-Luna et al., 2009), the enzymatic and metabolic profiles of soil microorganisms in the 0–15 cm layer was determine at a site where charcoal had been produced, in an undisturbed forest soil and in a transition zone between the two in the dry and rainy season. The objective of this study was to determine the impact of charcoal production on enzymatic and metabolic profiles of soil microorganisms at a KILN site in *Quercus* spp. temperate forest in central Mexico during the dry and rainy season.

2. Materials and methods

2.1. Study site location and soil characterization

The study area is located in Santa Rosa in the state of Guanajuato, Central Mexico (N.L. 20°50′59′ï–21°55′05″ and W.L. 100°59′28″–100°33′09″). The altitude of the study area is 2660 m above sea level, with mean annual temperature ranging from 12 to 18 °C and an average rainfall of 1100 mm. The rainy season starts in June and ends in September, while the driest part of the year is between March and May (CONABIO, 2007). The temperate forest covers 1486 km^2 in the state of Guanajuato and is dominated by *Quercus rugosa* Neé, the main species used for charcoal production.

The KILN sites were prepared in 1999 and used for one year. A KILN site of approximately 7 m in diameter was prepared by clearing all vegetation, removing the organic residue and litter (Oi and Oe horizon) from the surrounding area and flattening the soil. The wood was piled up in the centre of the KILN site, covered with litter, lit and then covered with soil. Some airing points at a high of 1.5 m were left uncovered and the incomplete combustion with a minimum of flame production was maintained for 12 to 14 days (Vázquez-Marrufo et al., 2003). The temperature of the covering soil varied between 200 °C and 300 °C. The soil was charred or carbonized to a depth of 20 cm and some of the charcoal produced was left at the site as waste product.

Three different locations at a distance of 50–70 m were identified within the same stand of approximately 1 ha. Three different points were sampled at each treatment and location: (i) non-disturbed soil that was never used to produce charcoal (the CONTROL treatment), (ii) soil where charcoal was produced for one year in 1999 (the KILN treatment), and (iii) a transition zone located between the area where charcoal was produced and the control soil. In the transition zone, the vegetation was cleared, but the litter layer was retained (the TRANSITION ZONE treatment). Soil was sampled from the 0–15 cm layer from the CONTROL, KILN and TRANSITION ZONE treatment in the dry (May) and rainy (July) season. As such, the number of soil samples obtained from each treatment in the dry and rainy season was nine ($n = 9$), but only soil sampled in the rainy season was used for characterization. Characteristics of the three sampling sites can be found in Tables 1 and 2.

The Oi and Oe horizons had been removed for the preparation of the KILN, but some humus (Oa horizon) remained at the KILN site. A layer of litter and humus was found in the TRANSITION ZONE and CONTROL treatments. The soils in the area are classified as haplic phaeozems (PHh) according to FAO/UNESCO (1988).

Table 1
Selected soil properties at the KILN sites (KILN treatment) and boundary sites (TRANSITION ZONE treatment) compared to an undisturbed forest with *Quercus* spp. as the dominant species (CONTROL treatment) at Santa Rosa (Guanajuato, Mexico).

Treatment	pH_{H_2O}	Organic C (g kg^{-1} soil)	Total N (g kg^{-1} soil)	Sand (g kg^{-1} soil)	Clay (g kg^{-1} soil)	Silt (g kg^{-1} soil)	USDA soil texture classification
KILN	7.0 (0.2)[a] A[b]	55.7 (0.7) B	5.1 (0.6) B	640 (30) A	110 (30) A	250 (40) A	Sandy loam
TRANSITION ZONE	5.4 (0.2) B	64.4 (0.7) B	4.8 (0.6) B	700 (30) A	70 (20) A	230 (20) A	Sandy loam
CONTROL	4.5 (0.1) C	114.0 (0.6) A	6.7 (0.2) A	730 (60) A	50 (10) A	220 (50) A	Sandy loam

[a] Value between parenthesis is the standard error of the estimate.
[b] Values with the same letter are not significantly different between the treatments ($P < 0.05$) ($n = 9$).