Applied Soil Ecology 83 (2014) 230–237

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Earthworm communities and soil properties in shaded coffee plantations with and without application of glyphosate

José Antonio García-Pérez[a,*], Enrique Alarcón-Gutiérrez[b], Yareni Perroni[b], Isabelle Barois[c]

[a] Facultad de Biología, Universidad Veracruzana, Circuito Gonzalo Aguirre Beltrán s/n, Zona Universitaria, C.P. 91090 Xalapa, Veracruz, Mexico
[b] Instituto de Biotecnología y Ecología Aplicada (INBIOTECA), Universidad Veracruzana, Av. de las Culturas Veracruzanas No. 101 Col. Emiliano Zapata, C.P. 91090 Xalapa, Veracruz, Mexico
[c] Instituto de Ecología, A.C., Carretera antigua a Coatepec 351, El Haya, Xalapa 91070, Veracruz, Mexico

ARTICLE INFO

Article history:
Received 17 January 2013
Received in revised form 13 September 2013
Accepted 16 September 2013
Available online 12 December 2013

Keywords:
Pontoscolex
Amynthas
Mineralization
Infiltration
Herbicide
Bioindicator

ABSTRACT

In central Veracruz, Mexico, many coffee plantations are managed using agrochemicals for weed control, with glyphosate-based herbicides (GBH) the most commonly used. To date, however, no studies in this region have characterized the soil biological and physicochemical properties in coffee plantations under such glyphosate application. In this study, earthworms were used as bioindicator organisms by measuring differences in the earthworm community in plots within shaded coffee plantations, with and without repeated applications of glyphosate. Differences in earthworm-induced soil processes, such as water infiltration rates, potential net carbon mineralization rates and soil physicochemical properties were also evaluated. Eight plots were selected in shaded coffee plantations; four had received regular applications of GBH over the preceding 22 years, while the other four had received no herbicides over the preceding 7 years. The earthworm species found in plots with no GBH treatment were *Pontoscolex corethrurus* (99%) and *Amynthas corticis* (1%), while *A. corticis* was absent in plots that had been treated with GBH. Significant differences ($P<0.01$) in earthworm density (168 ± 16 and 353 ± 37 ind m^{-2}) and biomass (22.7 ± 1.1 and 45.4 ± 6.9 g m^{-2}) were observed in soils with and without GBH, respectively. No significant difference ($P=0.08$) was observed in the water infiltration rate ($2 \times 10^{-4} \pm 4 \times 10^{-5}$ and $4 \times 10^{-4} \pm 1 \times 10^{-4}$ cm s^{-1} with and without GBH, respectively). Soil carbon flow was greater in plots with GBH (76 ± 7 μg dry soil^{-1} d^{-1}) than in those without GBH (62 ± 1 μg dry soil^{-1} d^{-1}, $P<0.005$). Significant differences ($P<0.05$) were found in pH and in the clay, silt and Ca content of the soil. Our findings indicated reduced species number, density and biomass of earthworms, and increased net carbon mineralization rate in plots with GBH. The plots managed with glyphosate presented a negative effect on the earthworm parameters measured, and we conclude that the earthworms therefore acted as indicators of perturbation. It is also possible that this effect could be due to factors unrelated to the glyphosate that were not considered in this study, such as chemical fertilization or legume litter spatial variability, among others.

1. Introduction

In Mexico, about 15,785 Mg of herbicides are applied to agricultural soils each year (INE, 2000; Raymundo-Raymundo et al., 2009). Of these herbicides, glyphosate is one of the most commonly used (Bejarano-González, 2002). Glyphosate is recommended by the companies Monsanto and Cheminova for weed control in coffee plantations and, while an average half-life of 2 to 174 days has been documented for glyphosate in soil (Cox, 1998, 2004), there is evidence that the non-labile phase of glyphosate can persist from 222 to 835 days (Al-Rajab et al., 2008; Eberbach, 1998). Coffee plantations cover about 22,900 ha (17% of the territory) in the state of Veracruz, Mexico (Muñoz-Villers and López-Blanco, 2008). They are located within an altitudinal range from 900 to 1300 m asl and thus overlap with the distribution area of tropical montane cloud forest (Manson et al., 2008). While small-scale coffee producers use more labor, it has been established that they apply the same amount of herbicide per hectare as applied by large-scale farmers (Bellamy, 2011). Some studies have shown that glyphosate has practically no immediate direct effects on non-target organisms such as invertebrates e.g. arthropods and oligochaetes (Buch et al.,

* Corresponding author.
Tel.: +522288184125/+1 228 842 17 48x11748x11617x11618, 11619; fax: +1 228 817 92 02.
E-mail addresses: garci95@hotmail.com, antoniogarcia01@uv.mx (J.A. García-Pérez).

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http://dx.doi.org/10.1016/j.apsoil.2013.09.006

2013; Dalby et al., 1995; Guiseppe et al., 2006; Lindsay and French, 2004; Santos et al., 2011). Other studies have indicated some negative impacts on these organisms, in terms of reduced abundance and other sub-lethal effects (Benamú et al., 2010; Casabé et al., 2007; Correia and Moreira, 2010; Haughton et al., 1999; Springett and Gray, 1992; Schneider et al., 2009; Verrell and Buskirk, 2004), while others show that invertebrate density is actually stimulated by glyphosate (Lins et al., 2007). Similarly, while some studies show the toxic effects of glyphosate on soil micro-organisms and microbial activity (microbial biomass, dehydrogenase and β-glucosidase; Tejada, 2009), others indicate stimulation of bacteria, enzymatic activity and microbial respiration (Lane et al., 2012; Means et al., 2007; Ratcliff et al., 2006). The evidence indicates that the persistence of glyphosate and its effect on soil biological activity is dependent on soil characteristics (mainly pH, texture and organic matter content; Albers et al., 2009; Pessagno et al., 2008; Tejada, 2009). Local coffee farmers report that the soil is becoming compacted and bare; however, studies that characterize soils under glyphosate application in the coffee plantations are practically non-existent.

In order to address this gap in the knowledge, we quantified earthworms and the processes they induce in soil that has been treated with glyphosate and compared these data to those measured in non-treated soils. Earthworms can be a very important component of soil ecosystems; they represent up to 80% of the total invertebrate biomass and can incorporate toxic substances through their skin (Hobbelen et al., 2006; Lord et al., 1980) or by the consumption of large amounts of soil (Lavelle and Spain, 2001; Yasmin and D'Souza, 2007). For these reasons, earthworms have been proposed as bio-indicator organisms of the impacts of pollutants (Buch and Brown, 2010; Paoletti et al., 1998; Paoletti, 1999); specifically, the density, biomass and diversity of these organisms have been suggested as indicator variables in field studies (Paoletti, 1999). Such effects on earthworms would have consequences for certain soil processes, given the strong relationship between earthworms and soil processes and properties, such as infiltration (Alhassoun, 2009; Capowiez et al., 2009; Fischer et al., 2013; Pitkänen and Nuutinen, 1998; Wuest, 2001), soil C and N mineralization (Bernard et al., 2012; Ganihar, 2003; Lachnicht et al., 2002; Lubbers et al., 2011; Steinberg et al., 1997; Subler et al., 1998; Willems et al., 1996) and bulk density (Zund et al., 1997).

We hypothesize that if glyphosate affects earthworm and soil processes, we would detect differences between contiguous coffee plots with or without the systematic application of GBH, particularly in terms of (i) earthworm diversity, density and biomass, and (ii) earthworm-induced changes to soil properties such as soil bulk density, soil water infiltration and soil net potential mineralization carbon rate.

The aim of this study was therefore to test these predictions by characterizing and comparing shaded coffee plots that have undergone regular glyphosate application (for the last 22 years) and those in which glyphosate application has been suspended for 7 years.

2. Materials and methods

2.1. Study area and site description

The study area is found in the "ejido" (communal land) of San Marcos de León, in the municipality of Xico, in Veracruz state, Mexico (19°42′27″ N: 96°96′36″ W). The climate is semi-warm, temperate, humid, with an annual average temperature of 19 °C and annual average rainfall of 1750 mm. The area is at an altitude of 1100 m asl with slight slopes (3%) (Gómez, 1991; Hernández-Hernández, 2010). The original vegetation was cloud forest, growing on andosols derived from volcanic ash (Meza-Pérez and Geissert-Kientz, 2006). Ejidal lands cover an area of 872 ha, of which 85 correspond to urban area while the coffee-cultivated area comprises around 787 ha (SEDATU, 2012). This area is cultivated in its entirety with different varieties of *Coffea arabica*.

In the ejido called "Tlalcontla", eight-shaded coffee plots (≈1 ha) were selected for the study. Six of these were contiguous, while the other two were located approximately 200 m apart from each other. Five plots were relatively flat (0°–10°) while three presented a slight slope (25°) within a small part of each plot. All of the coffee plots were classed as commercial polyculture, according to Moguel and Toledo (1999). The plots were shaded mainly with leguminous Fabaceae species of the genus *Inga* (Chalahuite). These trees form the arboreal cover of polyculture plots where coffee, citrus, black cherry and banana are grown together. Commercial formulations of glyphosate-based herbicides (GBH) were applied in the coffee plantations at least three times per year; in four of the eight study plots, this treatment has been carried out for the last 22 years, with Glyfos®, Classical Faena® and Faena Fuerte®. The other four plots, however, had been free of herbicides for seven years (following 15 years of GBH application). The study was carried out over one sampling period at the end of February 2011.

2.2. Sampling design

Three sampling points were randomly distributed within each plot, in a systematic sampling design comprising a total of 24 samples. The coffee plants in each plot were mapped and numbered, then used as a grid for sampling point randomization. The first sampling point was randomly generated and the remainder located in an equidistant zigzag pattern. Restrictions were imposed on the location of sampling points in order to avoid edge effects.

2.3. Earthworm sampling

The earthworm community was extracted from a soil monolith (25 × 25 × 30 cm; Anderson and Ingram, 1993) taken from each sampling point. Identifiable and visible earthworms and earthworm cocoons were manually sorted and preserved in alcohol (70%). In the laboratory, all the earthworms were identified to species, grouped into exotic or native and classified according to ecological category (Bouché, 1977). Density and biomass of the earthworms were recorded and the data extrapolated to produce a value per m^2.

2.4. Soil sampling and physicochemical analysis

Soil was collected from the monoliths and a composite soil sample (2 kg) generated by combining three subsamples from each plot. Prior to analysis, soil samples were refrigerated in the dark and then air-dried and sieved through a 2 mm mesh. Total carbon and total nitrogen (N) were determined by dry combustion using a LECO TRUSPEC auto-analyzer (LECO Corporation, 2002). Organic matter content was estimated from the total carbon results. Exchangeable bases were measured by extraction with ammonium acetate 1 N, at pH 7, followed by quantification using atomic absorption (for Ca and Mg) and flame spectrophotometer (for K and Na). Micronutrients (Fe, Cu, Mn and Zn) were extracted following the DTPA (diethylenetriamine-pentaacetic acid) method and then determined by atomic absorption. Nitrates (NO_3), ammonium (NH_4) and total phosphorous (P) were determined using the colorimetric method (Cataldo et al., 1975; Allan, 1971 in Etchevers, 1984). Available phosphorous (P) was determined with the Bray's method and pH measured with a potentiometer (ratio 1:2 soil:water). Texture was determined from particle size analysis using the Bouyucos hydrometer method (Page et al., 1982), real density by the pycnometer method and moisture content by the gravimetric method. All tests, except those for organic matter and N, were performed