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DYNAMICS OF BIOCHEMICAL SOIL PROPERTIES IN RAINFED *AGAVE ANGUSTIFOLIA* HAW. FIELDS IN SEMIARID ZONE

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Abstract

This study determined (a) the activity of the selected enzymes of C (invertase and cellulase), N (urease), P (acid phosphatase, AcP and alkaline phosphatase, AlkP) cycling, as well as soil microbial biomass carbon (SMBC) in the rhizosphere of *Agave angustifolia* Haw. (maguey espadín) cultivated in valley, hill and mountain soils in Tlacolula, Oaxaca (Mexico), and (b) changes in selected biochemical soil properties associated with seasonality and plant age. Three maguey espadín plantations with two replicates per topography were selected. Rhizosphere soil sampling was carried out for one year. AcP and urease activities were highest in mountain sites. AlkP and cellulase activities were highest in hill and mountain sites. Invertase activity and SMBC were not different among sites. Phosphatase activity showed no temporal changes. Cellulase and invertase activities were highest in December; maximum urease activity was in October and February. SMBC was highest in August. AcP activity was highest in 0-2 years old plants. AlkP activity and SMBC were highest in 2.1-4 years old plants. Urease, cellulase and invertase activity was not affected by plant age. Significant effects for the site×seasonality×plant age interaction suggest that biochemical soil properties were influenced by site. Mountain soils displayed the highest enzyme activity, which can accelerate soil organic matter turnover and contribute to the long-term sustainability of maguey espadín cropping under rainfed conditions.

Key words: Maguey espadín, Mountain soils, Plant age, Seasonality, Soil enzyme activity.

Introduction

The measurement of biochemical soil properties such as soil hydrolases activity provides an early indication of changes in soil fertility, as they are related to the mineralization of nutrients such as N, P and C (Trasar-Cepeda *et al.*, 2008). Phosphatases catalyze the hydrolysis of P from phosphate-monoesters into different forms of inorganic P, thereby enhancing plant P uptake (Balemi & Negisho, 2012). Urease catalyzes the hydrolysis of urea to CO_2 and NH_3; its origin is essentially microbial, and its activity is largely extracellular (Mohammadi, 2011). Invertase catalyzes the hydrolysis of sucrose to D-glucose and D-fructose and plays a critical role in releasing low-molecular-weight sugars, which are important energy sources for microorganisms (Jin *et al*., 2009). Cellulases are hydrolases that decompose cellulose (the most abundant polysaccharide in plant cell walls), compounds present in fresh plant residues that are continuously deposited above soil in the litter layer (Mtui, 2012). Soil microbial activity is influenced by numerous factors, such as C input, seasonal changes, hydric and thermal soil regimes (Aon *et al*., 2001).

In the rainfed semiarid area of Oaxaca State in Southern Mexico, approximately 15,000 ha have been cropped with *Agave angustifolia* Haw. for at least 100 years. *A. angustifolia* is colloquially referred to as "maguey espadín" and is used as a basic ingredient in the production of mezcal, a traditional alcoholic beverage in Oaxaca. In a previous study, Bautista-Cruz *et al.* (2007) reported the fertility conditions of soil cultivated with maguey espadín in the Tlacolula district of Oaxaca in accordance with topographic variations from agroecosystems and plant age, concluding that, in general, these soils are poor in terms of organic matter, nitrogen and phosphorus. In a later study, Bautista-Cruz *et al*. (2011) identified soil organic carbon, pH, soil microbial biomass carbon (SMBC), and exchangeable Mg^{2+} as potential indicators for a minimum data set for soil quality assessment in maguey espadín fields. The objectives of the present study were (a) to determine the activity of the selected enzymes of C (invertase and cellulase), N (urease), P (acid phosphatase and alkaline phosphatase) cycling as well as SMBC in the rhizosphere of maguey espadín cultivated in valley, hill and mountain soils, and (b) to explore the changes in the selected biochemical soil properties associated with the seasonality and plant age. Following the method proposed by Jin *et al.* (2009), the criteria for choosing enzyme assays was based on their importance in nutrient cycling and organic matter decomposition, and the simplicity of the assay. This study represents a first reference on the soil enzyme activity of a large area cropped with maguey espadín under rainfed conditions in a semiarid zone in Mexico.

Materials and Methods

Study sites: The area of study (16° 94' N and 96° 54' W) is located in the Tlacolula District of Oaxaca, Mexico. The location sites are San Baltazar Guelavila, San Juan del Río and Santiago Matatlán (Fig. 1). According to the soil survey reported by the National Commission of Biodiversity (2010) on the scale of 1:4,000,000, the main soil classes where maguey espadín is grown are located at an altitude of 1,060-1,700 m, and correspond to Regosols and Leptosols. The parent material is limestone rock with

lutite from lower Cretaceous (Castillo & Castro, 1996). Average annual rainfall is 726 mm; average annual temperature ranges from 28 to 32ºC. The climate is temperate-semiarid (National Commission of Biodiversity, 2010); the vegetation type is dry deciduous lowland forest (see Bautista-Cruz *et al*., 2007 for details of the study sites), where the dominant species are *Acacia* spp., *Bursera* spp., *Ipomea* spp., *Leucaena esculenta* and *Prosopis laevigata*. The cropping of this agave species has taken place for more than 100 years in the Tlacolula district.

Maguey espadín is cultivated in mountain zone (San Juan del Río) with minimum tillage; hills (San Baltazar Guelavila) with animal-drawn ploughing; and valleys (Santiago Matatlán) with disk ploughing. For each topography three maguey espadín plantations with the following plant ages were selected: 0-2 years, 2.1-4 years and $\geq$ 4 years, with two replicates from each site. Maguey espadín reaches sexual maturity between 7 and 10 years after being planted. During the harvesting process of maguey espadín, plant leaves are cut, chopped and left in the field, where they are eventually reincorporated into the topsoil. Fallow periods are not frequently employed in maguey espadín production. According to interviewed landholders, herbicides and fertilizers have never been applied to any of the plantations sampled in this study. However, distillery effluents are normally incorporated into the soil in all cases.

Soil sampling, processing and analysis: Soil sampling was conducted from August to December (rainy season) and from February to June (dry season) during 2011, following the procedures described by Boone *et al*. (1999). One representative plot of 4,000 m^2 was determined within each site, including the above mentioned plants. In each plot, five maguey espadín plants were selected for sampling; the first was field-centered and the others were at a distance of 25 m from the central maguey espadín plant, along the four cardinal directions. For each of the maguey espadín plants, we took four subsamples of rhizosphere soil including root fragments at 0-20 cm depth. A total of 20 rhizosphere soil subsamples were collected from each plot; these soil subsamples were mixed to form one composed rhizosphere soil sample per plot.

Following Zornoza *et al*. (2006) and Zornoza *et al*. (2007), soil enzyme activities, SMBC and chemical properties were measured in air-dried and sieved (< 2 mm) soil samples. Soil chemical properties such as plant-available P (P_{Olsen}), soil organic carbon, total N and soil pH were determined using standard chemical methods for soil analysis. Soil microbial biomass carbon was estimated via the fumigation-incubation method described by Jenkinson & Powlson (1976). Acid phosphatase and alkaline phosphatase activity were determined according to Tabatabai & Bremner (1969). The soil sample was incubated with a substrate containing *p*-nitrophenyl; the amount of *p*-nitrophenol liberated during enzymatic hydrolysis was measured by spectrophotometry. Invertase activity was determined with saccharose as the substrate. The soil sample was incubated at 37°C for 5 h. The liberated reduced sugars were determined by the method described by Nelson (1944). The same incubation conditions were used in determining carboxymethylcellulase activity, with the exception that the substrate was carboxymethylcellulose and the incubation time was 24 h (Pancholy & Rice, 1973). For urease activity determination, we used the Kandeler & Gerber (1988) method. After the addition of an aqueous (controls) or a buffered urea solution (samples) to five grams of soil samples, those were incubated for 2 h at 37 °C. Released ammonium was extracted with potassium chloride solution, and determined by a modified Berthelot reaction (Schinner *et al*., 1996). Controls were performed in all cases by adding the substrate after the reaction was stopped and before filtration of the soil suspension. Throughout soil analyses, 10% of the soil samples were randomly replicated for quality control.

Statistical analysis: The data was submitted to a two-way analysis of variance (ANOVA) and a multiple range test for mean separation (Tukey $p<0.05$). A Pearson correlation analysis was used to determine the relation between the analyzed variables. In all cases the statistical software JMP 7.0® (JMP 7.0 for windows 7) was used.

Results

Soil pH, soil organic carbon and total N content varied among sites. The soil pH value in the maguey espadín rhizosphere from the three sites ranged from moderately acid to moderately alkaline. This soil property decreased in the following order: hill > valley > mountain (Fig. 2). Soil organic carbon and total N were higher in rhizosphere soils of mountain and hill sites than in valley soils (Fig. 2). The three sites showed low levels of plant-available phosphorus, although the highest content of this nutrient was detected in valley soils (Fig. 2).

Acid phosphatase, alkaline phosphatase, urease and cellulase activities varied among sites. Acid phosphatase ranged from 53.08 µg p-NF g^{-1} soil h^{-1} to 115.77 µg p-NF g^{-1} soil h^{-1} and was highest in mountain soils (Fig. 3). Alkaline phosphatase activity ranged from 95.29 µg p-NF g^{-1} soil h^{-1} to 175.32 µg p-NF g^{-1} soil h^{-1}. The highest activity of this enzyme was found in hill and mountain soils, and the lowest was detected in valley soils (Fig. 3). Urease activity ranged from 5.25 µg of $N\text{-}NH^{+}_{4}$ g^{-1} soil h^{-1} to 6.98 µg of $N\text{-}NH^{+}_{4}$ g^{-1} soil h^{-1} and was highest in mountain soils (Fig. 3). Cellulase activity ranged from 0.070 µg of glucose g^{-1} soil 24 h^{-1} to 0.116 µg of glucose g^{-1} soil 24 h^{-1}, and was higher in mountain and hill soils than in valley soils (Fig. 3). Invertase activity ranged from 9.7 µg of glucose g^{-1} soil 5 h^{-1} to 10.75 µg of glucose g^{-1} soil 5 h^{-1}. Soil microbial biomass carbon ranged from 1687.8 µg C g^{-1} soil to 1798.5 µg C g^{-1} soil. The last two soil properties were not different among sites (Fig. 3).

The activity of both phosphatases showed no temporal changes over the sampling year. Cellulase and invertase activities displayed a high value in December and a low value in June (Fig. 4). Urease activity was high in October and February (early fall and mid-winter, respectively), but low in August (mid-summer) (Fig. 4). Soil microbial biomass carbon was highest in August (mid-summer, rainy season) and registered a low value in December and February (winter, dry season) (Fig. 4).

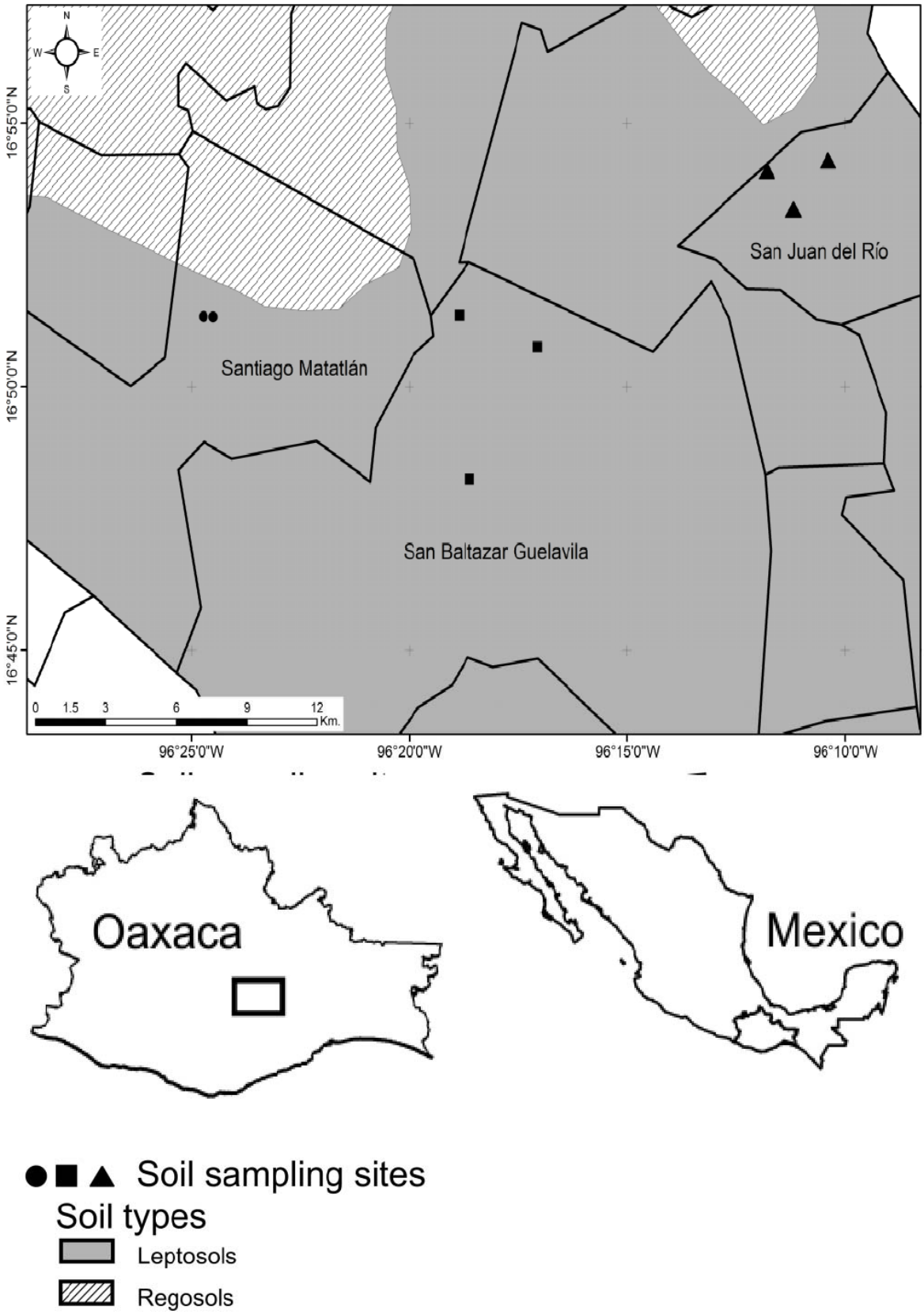

Fig. 1. Sampling sites in the Tlacolula District, Oaxaca, Mexico.

The average coefficient of variation for alkaline phosphatase activity was of 65.82%, 52.00% for acid phosphatase activity, 40.29% for urease activity, 63.02% for cellulase activity, 22.96% for invertase activity, and 43.13% for SMBC.

Acid phosphatase activity was highest in the rhizosphere soil of maguey espadín plants with an age of 0-2 years. In contrast, alkaline phosphatase activity was highest in the rhizosphere of maguey espadín plants with an age of 2.1-4 years (Fig. 4). Urease, cellulase and invertase activity was not affected by the age of agave plant (Fig. 5). Soil microbial biomass carbon was highest in the rhizosphere of maguey espadín plants with an age of 2.1-4 years (Fig. 5).

Significant effects for all the possible interactions among factors were found only for the site×seasonality×plant age interaction for acid phosphatase, alkaline phosphatase and urease activities, as well as SMBC. The highest acid phosphatase activity was detected in the rhizosphere of maguey espadín plants with an age of 0-2 years grown in mountain soils during dry season. The highest alkaline phosphatase activity was found in the rhizosphere of maguey espadín plants with an age of 2.1-4 years grown in hill soils during dry season. The highest urease activity was found in the rhizosphere of maguey espadín plants with an age of 2.1-4 years grown in mountain soils during rainy season. The highest SMBC was detected in the rhizosphere of maguey espadín plants with an age of 2.1-4 years grown in mountain soils during rainy season.

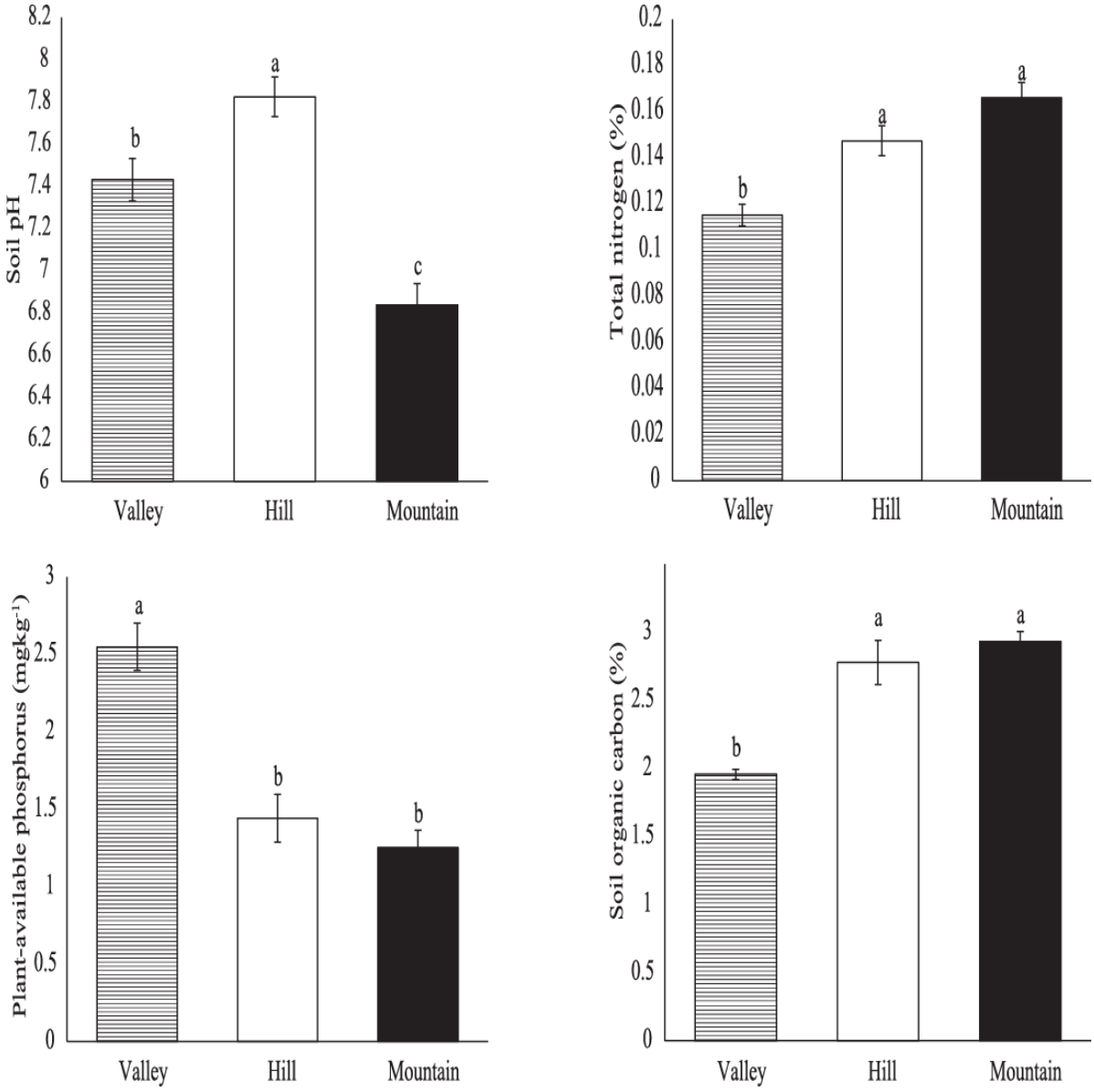

Fig. 2. Soil pH, total nitrogen, plant-available phosphorus and soil organic carbon content (mean ± standard error) in the rhizosphere of *Agave angustifolia* Haw. similar lower-case letters indicate no significant differences (Tukey test; $p<0.05$).

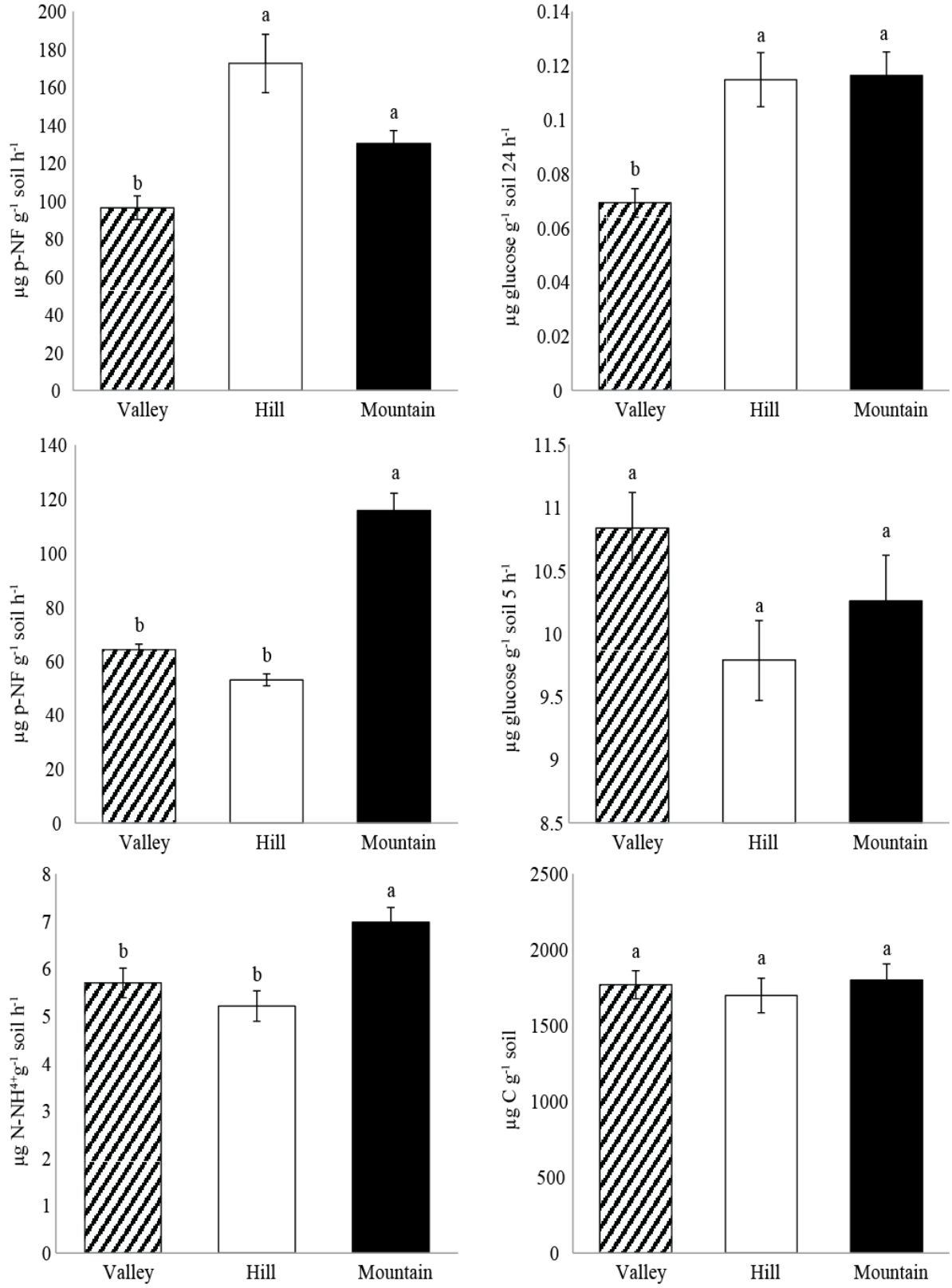

Fig. 3. Soil enzyme activities and soil microbial biomass carbon (mean ± standard error) in the rhizosphere of *Agave angustifolia* Haw. cropped in mountain, hill and valley soils in Tlacolula District, Oaxaca, Mexico. a) alkaline phosphatase, b) acid phosphatase, c) urease, d) cellulase, e) invertase and f) soil microbial biomass carbon. Similar lower-case letters indicate no significant differences (Tukey test; $p<0.05$).

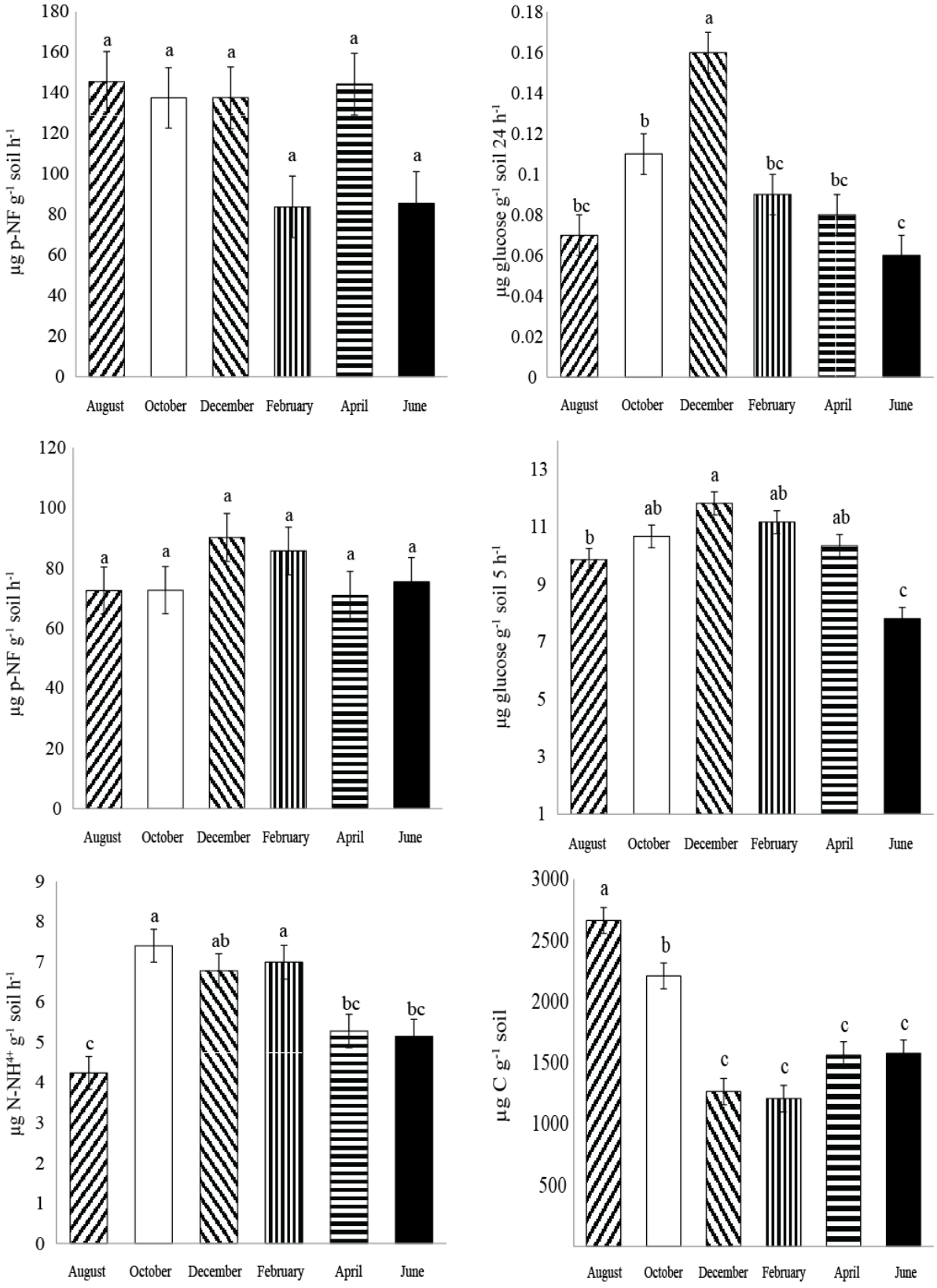

Fig. 4. Temporal changes of soil enzyme and soil microbial biomass carbon (mean ± standard error) in the rhizosphere of *Agave angustifolia* Haw. a) alkaline phosphatase, b) acid phosphatase, c) urease, d) cellulase, e) invertase and f) soil microbial biomass carbon. Similar lower-case letters indicate no significant differences (Tukey test; $p<0.05$).

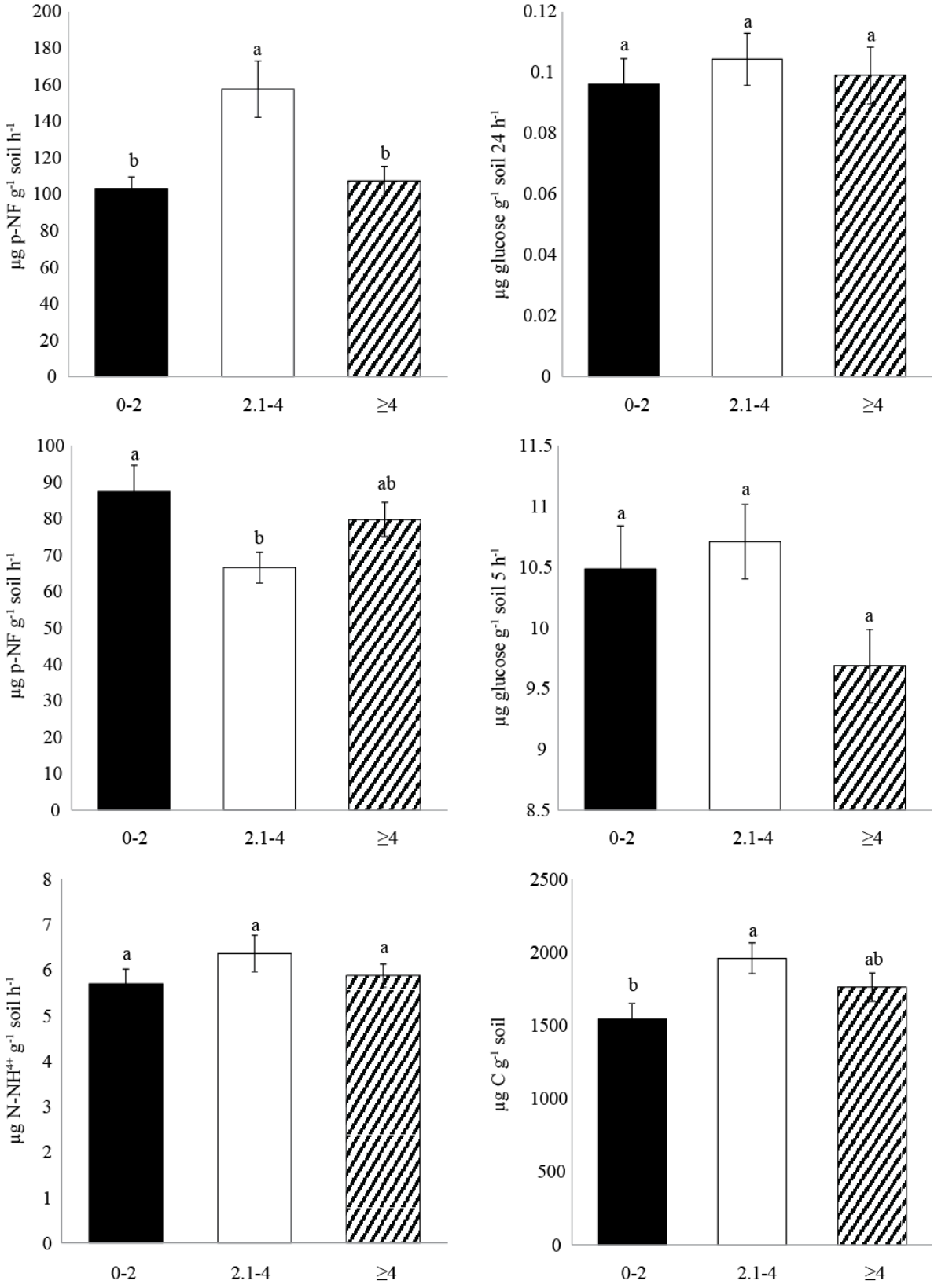

Fig. 5. Soil enzyme activity and soil microbial biomass carbon (mean ± standard error) in the rhizosphere of *A. angustifolia* associated with the plant age. a) alkaline phosphatase, b) acid phosphatase, c) urease, d) cellulase, e) invertase and f) soil microbial biomass carbon. Similar lower-case letters indicate no significant differences (Tukey test; $p<0.05$)

Discussion

The high acid phosphatase activity in the mountain soils is possibly due to the fact that these soils also had the highest soil organic carbon content. Phosphatase production and activity in particular, have been reported to be very sensitive to soil organic matter concentration (Goldstein *et al*., 1988). Acid phosphatase was highly associated with soil organic carbon (r = 0.243; p<0.002). Another factor which may contribute to explaining the high activity of this enzyme in the mountain soils is that these soils also had the lowest soil pH value, as acid phosphatase activity was closely and negatively correlated with soil pH (r = -0.633, p<0.0001). This correlation seems to indicate that acid phosphatase activity is affected by soil pH, which controls phosphorus availability in soil (Acosta-Martínez *et al*., 2007). Acid phosphatase, in addition to being affected by soil organic carbon, is also influenced by soil pH. Although a high level of alkaline phosphatase activity was found in hill soils, which also had the highest soil pH value, there was no correlation between this soil enzyme and soil pH in the study sites (r = 0.204; p>0.06). This result may be due to the interaction of other intrinsic soil properties that affect enzyme persistence and expression (Acosta-Martínez & Tabatabai, 2000).

The increase in urease activity in mountain soils could be associated with the high organic carbon content in this site. In this respect, Uzun & Uyanöz (2011) mentioned that urease activity is based on organic matter content and a decline in urease activity has been attributed to a decline in soil organic carbon content.

In general, the highest levels of enzyme activity were detected in mountain soils, which can accelerate soil organic matter turnover and benefit maguey espadín plant growth. In sites where plant residues remain on the soil surface, as is the case of mountain soils under minimum tillage, the high soil organic matter content provides substrate and energy for the soil biota. As a result, biological activity is stimulated, and consequently, enzymatic activity is also increased (Roldán *et al*., 2005).

Of the enzymes studied, acid phosphatase and alkaline phosphatase were more predominant than urease, cellulase and invertase. Other studies in semiarid soils (Acosta-Martínez *et al*., 2003) and humid soils (Klose & Tabatabai, 2002) have found that the glycosidases are less predominant than other enzymes. According to Tabatabai (1994), the predominance of soil enzyme activities is more related to the ecological role and kinetic characteristics of the enzymes studied than to the effects of the chemical and physical properties, geology, and land use of the studied soils. Phosphatases are typically the most abundant enzymes in the rhizosphere; their activity is related to the depletion of organic phosphorus (Kumari & Fulekar, 2011).

Soil microbial biomass carbon values were higher than those reported in agricultural semiarid soils (Srivastava, 1992) and those found in relatively dry tropical soils (290-450 μg C g^{-1} soil) (Srivastava, 1992; Srivastava & Lal, 1994).

Temporal variations in soil enzymatic activity are biologically important as they affect the quantity and quality of the substrates upon which they act and are responsible for altering the rate of various soil processes influenced by seasonal changes (Dkhar & Mishra, 1983). The activity of both of the phosphatases analyzed showed no temporal changes during the sampling year. In contrast, García-Ruiz *et al*. (2009) reported that acid phosphatase activity was relatively high during spring and early summer. However, alkaline phosphatase activity tended to increase from spring to fall. Grierson and Adams (2000) concluded that seasonal and spatial heterogeneity of the acid phosphatase activity of rhizosphere soil depended on plant species composition and root type (cluster roots or ectomycorrhizal roots).

Different results regarding temporal trends in cellulase activity were reported by Doyle *et al*. (2006). They found a higher cellulase activity in the summer than other seasons in soils of the Negev Desert. This result could be influenced by the cellulose source as well as by the quality of the cellulose incorporated in the soil. Furthermore, the decomposition of cellulose is controlled by cellulase levels in the soil (Wang *et al*., 2012). Debosz *et al*. (1999) reported the highest endocellulase activity in the autumn/winter and early summer in a field experiment after eight years of cultivation with either low organic matter input or high organic matter input.

Sardans *et al*. (2008) showed that soil urease activity was higher in winter than in summer. These results are concordant with those reported in this study. Akmal *et al*. (2012) determined the temporal changes in the activity of three soil enzymes in rainfed wheat and found the maximum urease activity in February which then decreased towards March and changed very little before May.

Yang *et al.* (2010) also found that the SMBC of forest soils was higher in summer than in spring and autumn. Changes in soil moisture, temperature and C input can also have a significant effect on soil microbial biomass and its activity, which, in turn, affects nutrient availability due to soil organic matter turnover (Ross, 1987).

The average coefficient of variation for alkaline phosphatase activity found in this study coincides with the variation coefficients of approximately 50% for acid and alkaline phosphatases, reported by Aon *et al.* (2001) during the growing season of a soybean crop in the Salado river basin. Our results also concur with those of Sinsabaugh *et al.* (2003), who found temporal coefficients of variation higher than 45% for soil enzymes in a sweetgum forest. Paz-Ferreiro *et al*. (2011) indicated a coefficient of variation higher than 30% for urease activity. Wardle (1998) reported coefficients of variation between 4% and 91% for soil microbial biomass. This indicates that the coefficient of variation of a biochemical soil property greatly depends on the property considered

and also on other factors such as type of climate, vegetation, and substrate (Paz-Ferreiro *et al*., 2011).

Previous studies have reported that plant age has direct influence on enzyme activity, especially on acid phosphatase activity (Li *et al*., 2002; Yadav & Tarafdar, 2004; Jin *et al*., 2009). Similarly, Yoshioka *et al*. (2006) found that both acid and alkaline phosphatase activity is influenced by the agronomic management and physiological state of the plantain crop. Soil microbial biomass carbon was highest in the rhizosphere of maguey espadín plants with an age of 2.1-4 years; this suggests that the rhizosphere conditions induced by the plant growth stage favor an increase in microbial biomass. Asghar *et al.* (2013) also reported the highest values of SMBC in the early stage of a wheat crop. The authors of the present paper concur with Mandal *et al*. (2007), who indicated that further studies are required to determine temporal changes in microbial communities in soil, and the functional significance of these changes during plant growth stages.

Significant effects for the site×seasonality×plant age interaction suggest that the dynamic of the majority of biochemical soil properties analyzed was influenced by the site. Dkhar & Mishra (1983) stated that temporal variations in the microbial population due to season, crop age and changes in soil physico-chemical properties, are not simultaneously reflected in the changed enzymatic activities. There may be a time lag between the physico-chemical changes and their corresponding changes in microbial population and activity.

Conclusions

The highest enzyme activity was found in mountain soils, which can accelerate soil organic matter turnover and benefit maguey espadín plant growth. Phosphatase activity showed no temporal changes. Cellulase and invertase activities were highest in December, whereas, maximum urease activity was in October and February. Soil microbial biomass carbon was highest in August. Acid phosphatase activity was highest in plants with 0-2 years age. Alkaline phosphatase activity and soil microbial biomass carbon were highest in plants with 2.1-4 years age. Urease, cellulase and invertase activity was not affected by the plant age. Significant effects for the site×seasonality×plant age interaction suggest that the dynamics of the majority of biochemical soil properties was influenced by the site.

The information generated in this study may be utilized for maintaining biological soil fertility and maguey espadín crop management in the rainfed region. More research is needed to further the study of the temporal variability of soil enzymes in maguey espadín fields. Other important aspects that need to be investigated are (i) the variation in the carbohydrate content of the maguey espadín grown in the three studied soil types, (ii) the effect of leaving maguey leaves (cut and chopped) in the fields during harvest on soil properties and, (iii) the influence of distillation waste on soil fertility and growth of maguey espadín.

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