quantity and quality of organic materials it provides. Quality is determined by the content of labile and recalcitrant compounds of the litter (C:N ratio); rapid decomposition indicates a higher concentration of labile components in relation to the recalcitrant (C:N low), which promotes the rate of decomposition and, therefore, leads to high respiration rates; and, directly, since the respiration of the roots is one of the main contributors to CO_2 (Hasset and Banwart, 1992; Raich and Tufekcioglu, 2000).

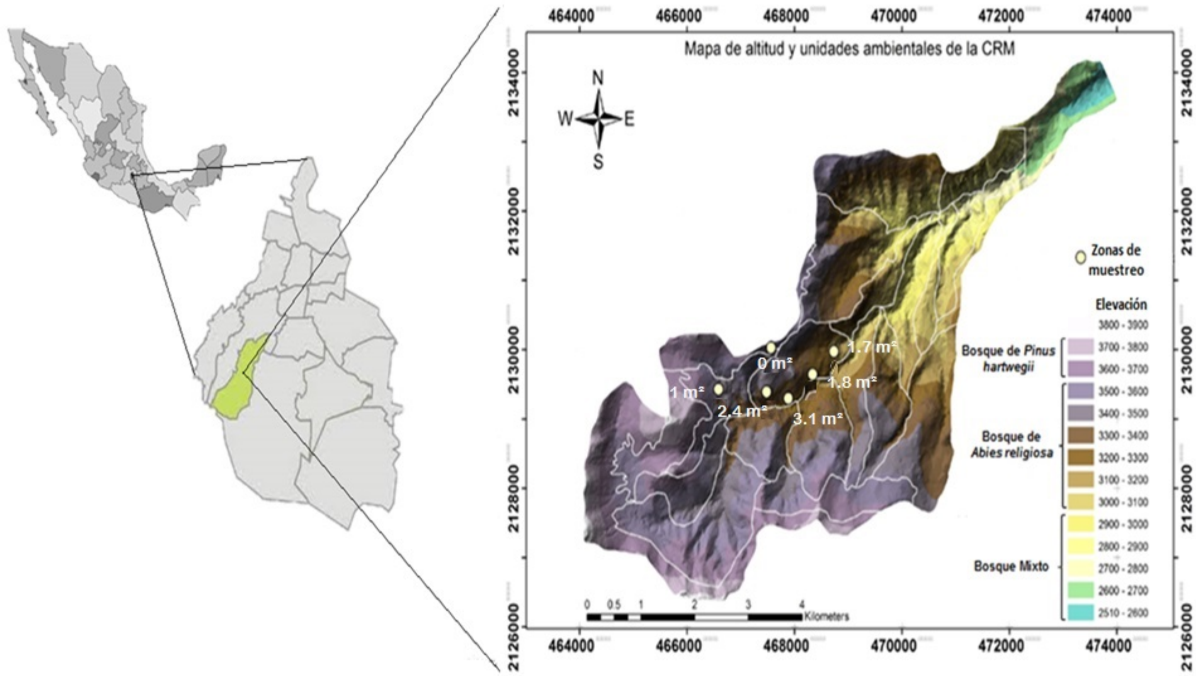
Since all these factors are modified by land use change in, it is interesting to assess their impact on the respiration of the soil biota. Currently, it is estimated that between 30 and 66 % of the national territory are under some agricultural or livestock use (Dupuy *et al*., 2012) and the loss of forest resources is between 500 000 and 631 000 ha per year (Díaz-Gallegos *et al.*, 2008).

Field studies that estimate respiration in Mexican soils are scarce (García *et al*., 2001; Campos, 2004; Covaleda *et al*., 2009; Campos, 2014) and laboratory tests (Álvarez and Anzueto, 2004; Ikkonen *et al*., 2004; Alcántara, 2009; Cruz *et al*., 2012; Cueva *et al.,* 2016), and none has been carried in the green areas of Mexico City. Therefore, the present work was carried out whose objective was to determine and contrast the microbial activity through breathing, in areas with different basimetric area in the *Abies religiosa* forest of the *Magdalena* river basin.

Materials and Methods

Study area

The study was carried out in the *Abies religiosa* forest belonging to the *Magdalena* River Basin (CRM, for its acronym in Spanish). It is a tall, evergreen forest with dense areas that reaches 100 % of vegetation cover and with the dominance of the tree and shrub strata, although there are also open areas due to disturbances and livestock. It is located on steep slopes, at 2 750-3 500 masl. In Figure 1, the sampling sites along the *Abies religiosa* forest appear in white and the basimetric area corresponding to each site is indicated.

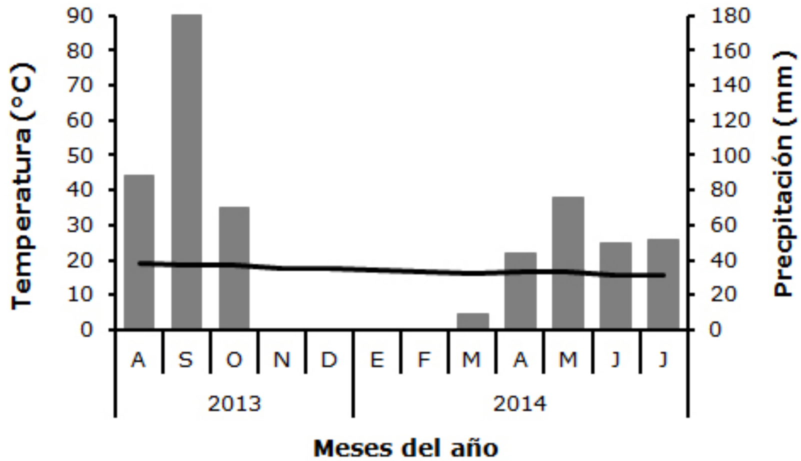


Source: modified from Santibáñez (2009).

Mapa de altitudes y unidades ambientales de la CRM = Map of altitudes and environmental units of the CRM; *Zonas de muestreo* = Sampling areas.

Figure 1. *Magdalena* river basin at the southeast of Mexico City.

The minimum rainfall recorded is 1 000 mm in the lower and maximum, and up to 1 500 mm in the highest peaks. There are two types of climates, from 2 400 to 2 800 masl, with a sub-humid climate with an average annual temperature between 12 °C and 18 °C. A semi-cold climate and an average annual temperature between 5 °C and 12 °C predominate from 2 800 to 3 800 masl (García, 1988). Figure 2 shows the temperature and precipitation data corresponding to the study period.



Source: *Desviación Alta al Pedregal* metheorological station.

Temperatura = Temperature; *Meses del año* = Months of the year; *Precipitación* = Precipitation.

Figure 2. Average temperature and total precipitation per month of the study period in the *Magdalena* river basin of Mexico City.

The soil in the forest of *A. religiosa* in the CRM is a humic Andosol (FAO-UNESCO) with medium silty texture to sandy loam (INEGI, 2006). The canopy reaches 20 to 30 m high; the shrub layer is 2 to 3 m high and a herbaceous layer is present. The following plant associations have been reported: *A. religiosa - Roldana angulifolia* (DC.) H. Rob. & Brettell and *A. religiosa - Acaena elongata* L. (Almeida *et al.,* 2007).

Selection of sites

The study was carried out in September 2013 (rainy season) and in May 2014 (dry season). Six sites with different vegetation cover were chosen (Table 1) where a 25 × 25 m square was plotted per site, in which the number of species and the perimeter at breast height (PAP) of *Abies religiosa* trees were recorded to obtain the basimetric area.

There were no trees in the most disturbed sites, the richness of herbs was 18 species and the most abundant was *Bromus carinatus* Hook. & Arn., which is a weed native to Mexico and is used as fodder (Rzedowski and Rzedowski, 2001); at the other end of the conservation gradient, the conserved site is located with the dominant presence of trees of *A. religiosa*. The site with the greatest richness (26 species) presented a basimetric area of 180 cm^2 and the dominant shrub was *Senecio angulifolius* DC. (Table 1), characteristic species of intermediate successional stages in temperate forests (Rzedowski and Rzedowski, 2001).

Table 1. Basimetric area of standing trees, vegetal richness and name of the most abundant species in each one of the sites of the *Abies religiosa* (Kunth) Schltdl.& Cham. forest in the *Magdalena* river basin of Mexico City.

Basimetric area (cm^2)	**0**	**100**	**170**	**180**	**240**	**310**
Vegetal richness	18	9	24	26	14	2
Dominant species	*Bromus carinatus* Hook. & Arn.	*Acaena elongata* L.	*Salvia gesneriflora* Lindl. & Paxton	*Senecio angulifolius* DC.	*Senecio angulifolius* DC.	*Abies religiosa* (Kunth) Schltdl. & Cham.

In the corner and center of each frame, a soil sample was collected at 10 cm deep with a 200-A Soilmoisture auger (10 cm high × 7 cm in diameter). Five replications per site were obtained in each of the seasons (rainy and dry). The samples were stored in a refrigerator at 4 °C until processing. At the same time, at each point a second unaltered soil sample was collected with a 100 cm^3 auger.

Preparation and analysis

The first five samples were screened with a 2.38 mm mesh opening to estimate bacterial and fungal respiration, and gravimetric moisture content. In the unaltered sample the apparent density, the concentration of carbon (C) and total nitrogen (N), as well as the pH were measured.

The C and N analyzes were performed in the Soil Fertility Laboratory of the *Colegio de Postgraduados* (Graduate College) (Colpos). Total nitrogen was determined by acid digestion with sulfuric acid and colorimetric analysis (Technicon Industrial Systems, 1977). Total C was determined by the modified Walkley-Black method (Technicon Industrial Systems, 1977).

To determine the apparent density, the unaltered soil sample was dried at 105 °C until it reached a constant weight (Siebe *et al*., 2006).

The pH was recorded using a PC18 Conductronic potentiometer (Hendershot *et al*., 2007).

The gravimetric moisture content was determined by weighing 50 g of soil collected in the field and placed in an ED53-UL#02-30777 *Binder* drying oven at 105 °C until reaching a constant weight (Jarrell *et al*., 1999).

Microbial respiration

The Isermeyer technique (Alef, 1995) was followed to measure the release of CO_2 by the total microbial biomass of the soil, which consists of placing a known weight of soil (not wetted) in a 100 mL beaker inside jars with a tight-fitting lid, in which a 0.05 M NaOH solution was added; these were placed in an incubator at 25 °C for three days. At the end of this period, 5 mL of 0.5 M barium chloride solution ($BaCl_2$) and four drops of indicator (0.05 M phenolphthalein solution) were added and the samples turned pink. Finally, the pink solution was titrated with hydrochloric acid (HCl) at a concentration of 0.05 M, until it became colorless. The amount of CO_2 was determined from the volume of HCl used (Alef, 1995).

The same technique was used for fungal biomass, but 85 % lactic acid ($C_3H_6O_3$) was added to eliminate bacterial populations, and thus, evaluate the release of CO_2 by fungi (Van Netten *et al*., 1994; Dubal *et al*., 2004; Wolf *et al*., 2012). To obtain the release of the bacterial CO_2, the fungal CO_2 was subtracted from the total microbial CO_2.

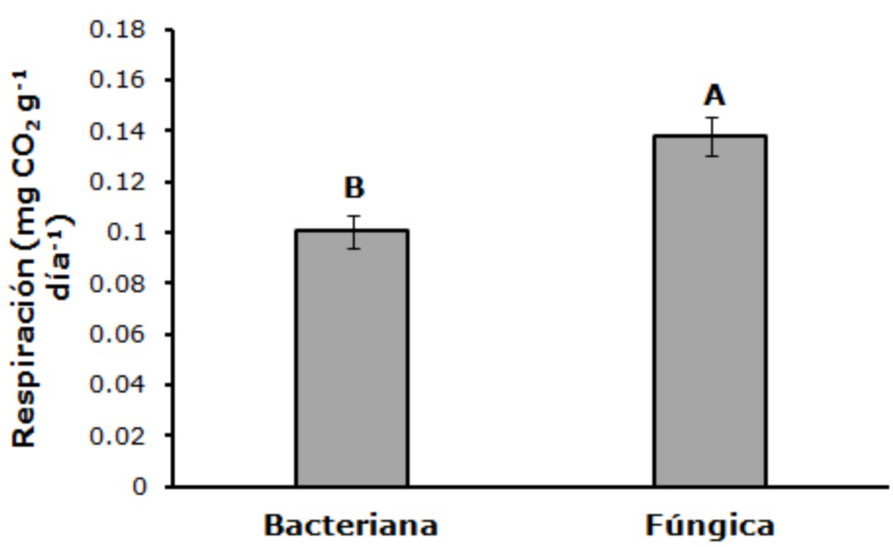
The control bottles had no soil, and with them the same procedure was performed.

Statistical analysis

For the analysis of the release of CO_2, an analysis of variance (complying with the assumptions of normality and homocedasticity) was performed in three ways considering as factors the source of CO_2 (bacterial, fungal), the site (basimetric area) and seasonality of rainfall, and when significant differences appeared, the Tukey test was performed. Linear regressions were also made between edaphic variables and CO_2 release. All analyzes were performed with 95 % confidence in the STATISTICA 8.0 statistical package (StatSoft Inc., 2007).

Results

Significant differences in CO_2 release were recorded between bacterial biomass and fungal biomass ($F_{(1,96)}$ = 138.34; P <0.0001). The potential release of CO_2 from the fungal biomass was superior to the bacterial one (Figure 3).

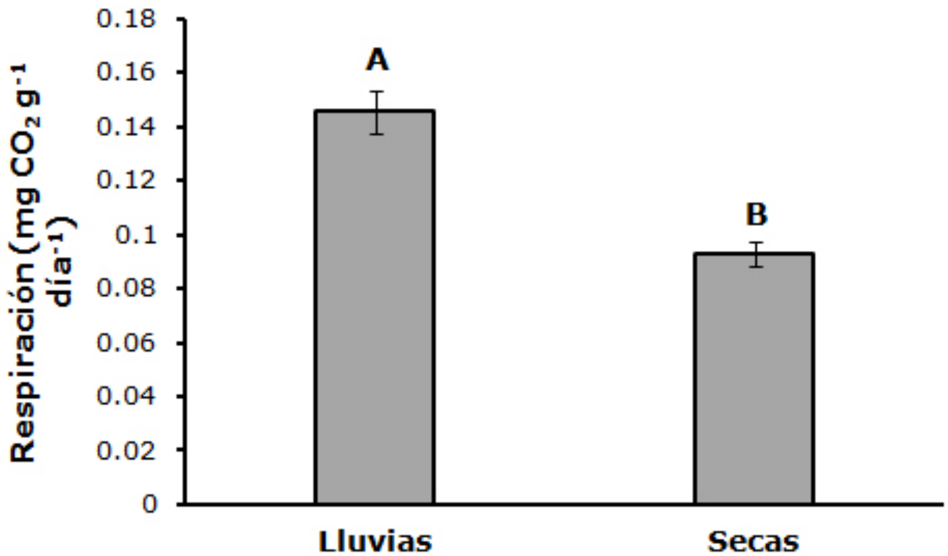


Different letters indicate significant differences (P < 0.0001).

Respiración = Respiration; *Bacteriana* = Bacterial; *Fúngica* = Fungi.

Figure 3. CO_2 potential release of the bacterial and fungi biomass in the *Abies religiosa* (Kunth) Schltdl. & Cham. forest in the *Magdalena* river basin of Mexico City.

The CO_2 release also showed significant differences between seasons ($F_{(1,96)}$ = 279.55; P <0.0001). There was 1.6 times greater breathing in the rainy season compared to the dry season (Figure 4).



Different letters indicate significant differences (P < 0.0001).

Respiración = Respiration; *Lluvias* = Rainy season; *Secas* = Dry season.

Figure 4. CO_2 potential release in the rainy season and in the dry season in the *Abies religiosa* (Kunth) Schltdl. & Cham. forest in the *Magdalena* river basin of Mexico City.

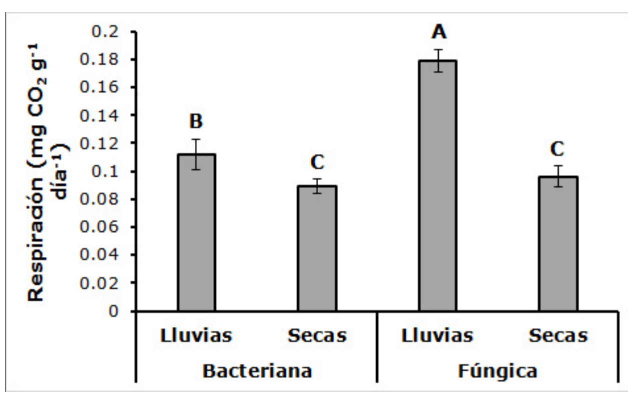
The CO_2 emission at the sites recorded significant differences ($F_{(5,96)}$ = 32.4; P <0.0001). The highest value was presented on the site with a basimetric area of 100 cm^2 and the lowest in that with 240 cm^2; there was a 40 % difference between these two sites (Table 2). At the site with the highest basimetric area (absence of undergrowth and herbaceous stratum), 13 % less CO_2 release occurred compared to the highest value.

Table 2. Potential release of microbial CO_2 (mean ± standard deviation) from six sites with different basimetric area in the *Abies religiosa* (Kunth) Schltdl. & Cham. in *Magdalena* river basin of Mexico City.

Basimetric area (cm^2)	CO_2 (mg CO_2 g^{-1} $día^{-1}$) Average
0	0.106 (±0.055) CD
100	0.155 (±0.072) A
170	0.116 (±0.072) C
180	0.108 (±0.033) CD
240	0.094 (±0.040) E
310	0.135 (±0.042) B

Different letters indicate significant differences (P < 0.0001).

The interaction between the source of CO_2 release and the season was significant ($F_{(1,96)}$ = 90.42; P <0.0001). The highest value corresponded to the combination of fungal biomass in the rainy season, and the CO_2 release was approximately double than recorded in the dry season in each of the groups of microorganisms (Figure 5).



Different letters indicate significant differences (P < 0.0001).

Respiración = Respiration; *Lluvias* = Rainy season; *Secas* = Dry season; *Bacteriana* = Bacterial; *Fúngica* = Fungi.

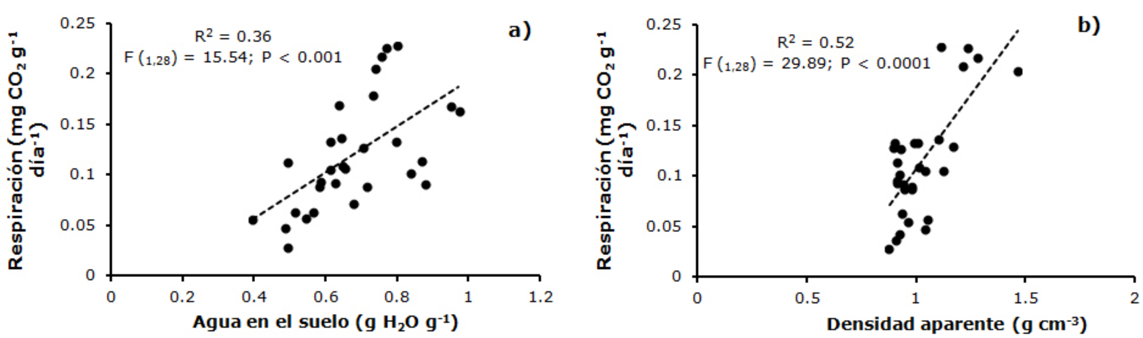
Figure 5. CO_2 potential release of the bacterial and fungal biomass in the rainy and dry season in the *Magdalena* river basin of Mexico City.

The interaction between the source of CO_2 release and sites with different basimetric area was also significant ($F_{(5,96)}$ = 34.94; P <0.0001). The lowest values corresponded to bacterial biomass at sites with 170 and 180 cm^2 of basimeric area, and the highest were at sites of 170 and 310 cm^2 in combination with fungal biomass.

Also, the interaction between season and sites with different basimetric area was significant ($F_{(5,96)}$ = 35.18; P <0.0001). In the dry season, the lowest value was found in the site with 240 cm^2 of basimetric area, while the highest corresponded to the site with a basimetric area of 100 cm^2 in the rainy season.

Finally, the interaction between the CO_2 release source, the season and the sites with different basimetric area was significant ($F_{(5,96)}$= 36.65; P <0.0001); the lowest average corresponded to bacterial biomass in the rainy season at the site of 170 cm^2 of basimetric area, and the highest to fungal biomass in rains at the site of 100 cm^2.

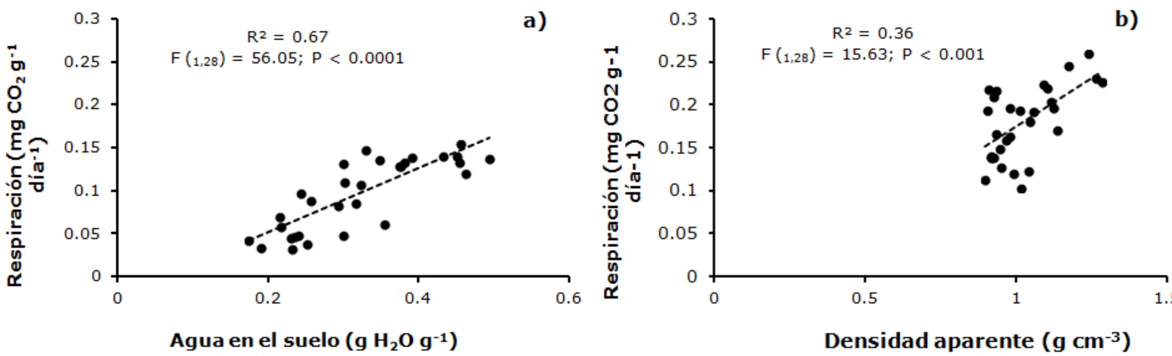
Although differences in CO_2 release between sites were detected, the relationship between the basimetric area and respiration was not significant, but there were linear relationships with some of the soil variables. The potential release of CO_2 from bacterial biomass was linearly and positively related to the gravimetric moisture content in the soil (R^2 = 0.36, $F_{(1,28)}$ = 15.54; P <0.001) and to the bulk density (R^2 = 0.52, $F_{(1,28)}$ = 29.89; P <0.0001) in the rainy season (Figure 6).



Respiración = Respiration; *Agua en el suelo* = Moisture content; *Densidad aparente* = Apparent density.

Figure 6. Relationship between the potential release of CO_2 from bacterial biomass and the gravimetric moisture content (a) and between the apparent density (b) in the rainy season, in the *Magdalena* river basin in Mexico City.

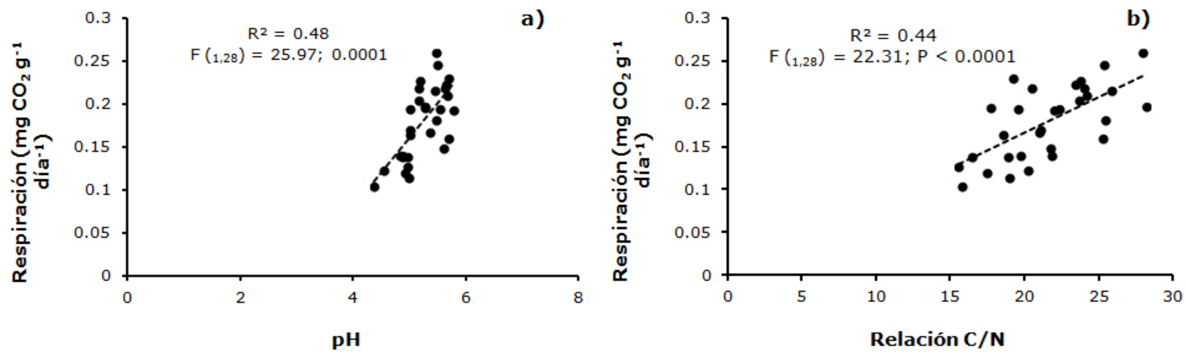
The release of CO_2 by fungal biomass was also linearly and positively related to the gravimetric moisture content in the soil during the dry season (R^2 = 0.67, $F_{(1,28)}$ = 33; P <0.0001) and with the bulk density of the soil in rains (R^2 = 0.55, $F_{(1,28)}$ = 33; P <0.0001) (Figure 7).



Respiración = Respiration; *Agua en el suelo* = Moisture content; *Densidad aparente* = Apparent density.

Figure 7. Relationship between the potential release of CO_2 from fungal biomass and the gravimetric moisture content in the dry season (a) and between the apparent density in the rainy season (b), in the *Magdalena* river basin in Mexico City.

Also in the rainy season, the release of CO_2 by the fungal biomass was linearly and positively related to the pH (R^2 = 0.48, $F_{(1,28)}$ = 25; P <0.0001) and the quality of the organic matter of the soil (estimated through the C: N ratio) (R^2 = 0.44, $F_{(1,28)}$ = 22; P <0.0001). The lowest respiration values are located at a pH near 4 and the highest at pH values close to 6; while the activity of fungal biomass increases at high values of the C/N ratio (Figure 8).



Respiración = Respiration; *Relación C/N* = C/N ratio.

Figure 8. Relationship between the release of CO_2 from fungal biomass and pH (a) and the C / N (b) ratio, in the Magdalena river basin in Mexico City.

Discussion

The recorded CO_2 values in this work are within the range of values for temperate forests: 0.01 to 0.70 mg CO_2 g^{-1} day^{-1} (Bailey *et al*., 2002; Ikkonen *et al*., 2004; Campos, 2014).

Although no relationship was found between the basimetric area and the potential release of CO_2, the site where a richness of 9 plant species was recorded, with the dominance of *Acaena elongata* and whose basimetric area is 100 cm^2 was the one that recorded the CO_2 highest values, followed by the site where there were only trees (310 cm^2) of *A. religiosa*, suggesting, as in other studies, that heterotrophic respiration rates are modified according to changes in vegetation structure during succession, which has been observed in both temperate and subtropical forests (Yan *et al*., 2009; Susyan *et al*., 2011).

In this study, the release of CO_2 by microbial biomass (bacteria and fungi) was different between sites, but did not follow an ascending pattern depending on the basimetric area or species richness (Table 2). Thus, the highest value of respiration, in addition to registering at a site with few plant species (9), also coincided with the dominance of *Acaena elongata*, a species that indicates disturbance and has a high demand for light, and which tends to grow in spots and dominate the herbaceous stratum (Rzedowski and Rzedowski, 2001), which prevents the establishment of other species.

In two of the sites with significant presence of trees (180 and 240 cm^2 of basimetric area) *Senecio angulifolius*, dominant species that reaches up to 5 m high, favors the entry and establishment of other species in the soil (Rzedowski and Rzedowski, 2001), which means that the sequence is in an intermediate stage; low CO_2 potential release values were recorded here. Instead, Susyan *et al.* (2011) observed that the basal respiration rates of the soil, as well as the microbial biomass increase during the succession process after the abandonment of agricultural land, which is attributed to an increase in the entrances and accumulation of organic C in soil.

In regard to the relationship between CO_2 release and some soil characteristics, a reduction in respiration rates due to an alteration of the soil structure, consistent with human activities within the CRM, was expected; in general, these tend to result in soil compaction, reducing the flow of oxygen and water along the soil profile (Tan, 2000), thus limiting microbial activity, so that the degree of compaction is reflected in high values of apparent density. In this regard, Torbert and Wood (1992) and Pengthamkeerati *et al.* (2005) showed a reduction between 60 and 65 % of soil CO_2 flows with values of apparent density in the range of 1.4 - 1.8 g m^{-3}. Additionally, Li *et al.* (2002) observed a decline between 26 and 39 % in the abundance of bacteria, fungi and actinomycetes in the soil as a reaction to an increase in bulk density. In this work the density values were in the range of 0.9 and 1.3 g cm^{-3}, which indicates that the soil has no compaction problems and that it has a good structure despite the different land uses that occur in the CRM.

The acid pH and a high content of recalcitrant materials favor the establishment of the fungal communities (Joergensen and Wichern, 2008; Paul, 2015; Kamble *et al.,* 2016), hence a positive and significant linear relationship between the CO_2 release from fungal biomass and these two variables. These results are consistent with those of Lou and Zhou (2006) and Zhang *et al*. (2016), who found that fungi are more resistant to acidic pH than bacteria in forest soils. However, the data in this study show a clear decrease in microbial respiration rates at pH values lower than 5. Sinsabaugh *et al.* (2008) recorded that very low pH values limit the enzymatic extracellular activity of fungi, which would explain what was found in this work.

The C/N ratio of the leaf fraction of the litter of *A. religiosa* is 50 (Barajas, 2014), which indicates that it is a substrate of difficult degradation, while the C/N ratio of soil organic matter in the rainy season goes from 20 to 25 and under dry conditions it reaches only values equal to 15. The highest CO_2 release was recorded at values of 25 in the C/N ratio of the organic matter of the soil, which was observed during the rainy season, so that a good quality, combined with the water present in the soil, favors microbial activity.

Several authors have observed a very marked response of microbial respiration rates in relation to seasonality, presenting the highest values during the rainy season, which is a consequence of a greater availability of nutrients and water in the soil and the consequent activation of the microbial community (Raich and Schlesinger, 1992; Yan *et al*., 2009; Wood *et al*., 2013). On the other hand, during periods of drought, access to nutrients becomes limited as the water layer inside the soil is reduced (Lou and Zhou, 2006; Barnard *et al*., 2013), resulting in lower decomposition rates and therefore breathing. In this work, approximately twice the release of CO_2 was recorded in the rainy season with respect to the dry season, which is consistent with what was reported in studies conducted in temperate ecosystems, in humid tropical and in transformed environments, such as crops and grasslands (Munson *et al.,* 2010; Carbone *et al*., 2011; Thomey *et al.,* 2011; Galicia *et al*. 2016).

The fact that there has been a positive linear relationship between the release of CO_2 and the moisture content in the soil during the dry season, could be due to the fact that the fungi have greater resistance to desiccation associated with their mycelial growth, which It allows spreading their hyphae along the pores of the soil absorbing nutrients and water (De Vries *et al.,* 2012; Paul, 2015). Likewise, fungi as growth strategies k produce more enzymes to access the use of more organic forms of C accumulated during this period (Waldrop *et al.,* 2006).

Conclusions

The results in this work indicate that the sites in a succession process in their early stages and that have less basimetric area in the CRM, generate a high potential release of CO_2 in the soil.

They also corroborate the relationship between some soil characteristics and microbial activity, the gravimetric moisture content, the pH, the apparent density and the quality of the organic matter modified the potential release of CO_2. So it is a multifactorial process.

Finally, it is suggested that disturbed areas should be recovered to reduce CO_2 emissions from the soil into the atmosphere of Mexico City.

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Conflict of interests

The authors declare no conflict of interests.

Contribution by author

Guadalupe Barajas Guzmán: field work, analysis of results and writing of the article; Dulce Hernández Rosales: field and laboratory work; Sally Paredes García: field and laboratory work; Juan Carlos Peña Becerril: field work and review of the manuscript; Javier Álvarez Sánchez: field work and review of the manuscript.

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