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Impact of anthropogenic activities on soil microbial biomass in a pre-montane forest in the foothills of the Andes Mountains.

Impacto de actividades antropogénicas sobre la biomasa microbiana del suelo en un bosque pre-montano de las estribaciones de la cordillera de los Andes

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ABSTRACT

Soil microbial biomass (BMS), CO_2 emissions, and organic carbon ($C_{organic}$) contents were quantified in soils with different anthropogenic uses in the "Bosque Protector Murocomba" (BPM). Five land use scenarios (treatments) were established (primary forest, secondary forest, fallow, plantation of *Gmelina arborea*, and pasture), within the Murocomba Protective Forest. Three soil samples were collected per treatment. Induced substrate respiration technique was employed using glucose as inducer, streptomycin and chloramphenicol to inhibit bacterial populations, cycloheximide and captan 80 as fungal inhibitors. The CO_2 released was trapped in NaOH solution (0.1 M) and titrated with HCl (0.1 M). Total $C_{organic}$ contents, active microbial biomass and CO_2 emissions were higher in the primary forest soil: 20.0 mg kg-1, 6.7 mg C-microbial g-1 dry soil (mg C-mic g-1 ss), and 50.4 mg CO_2 100 g-1 s hour-1. Grassland soils generated lower contents: 12.5 mg kg-1, 2.1 mg C-mic g-1 ss, and 15.9 mg CO_2 in 100 g-1 s hour-1, respectively. In all soils, fungal biomass predominated over bacterial biomass. These results demonstrate that the soils of the BPM are important reserves of organic C, however, anthropogenic activities generate changes in the dynamics of the BMS in these natural forests of the western foothills of the Andes, causing alterations in nutrient cycling. This research constitutes a baseline that places the BPM as a control point for future regional or global biogeochemistry studies.

Keywords: Temperatures, Modeling, Optimization, Tomato.

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RESUMEN

Se cuantificó la biomasa microbiana del suelo (BMS), emisiones de CO_2, y contenidos de carbono orgánico ($C_{orgánico}$), en suelos con diferentes usos antropogénicas en el "Bosque Protector Murocomba" (BPM). Se establecieron cinco escenarios (tratamientos) de uso del suelo (bosque primario, bosque secundario, barbecho, plantación de *Gmelina arbórea*, y pastizal), dentro del BPM. Se colectaron 3 muestras de suelo por tratamiento. Se empleó la técnica de respiración inducida de sustrato usando glucosa como inductor, estreptomicina y cloranfenicol para inhibir poblaciones bacterianas, cicloheximida y captan 80, como inhibidores fúngicos. El CO_2 liberado se atrapó en una solución de NaOH (0.1 M) y tituló con HCl (0.1 M). Los contenidos de $C_{orgánico}$ total, biomasa microbiana activa y emisiones de CO_2, fueron superiores en el suelo de bosque primario: 20.0 mg kg^{-1}, 6.7 mg C-microbiano g^{-1} de suelo seco (mg C-mic g^{-1} ss), y 50.4 mg CO_2 100 g^{-1} s $hora^{-1}$. Los suelos de pastizal generaron menores contenidos: 12.5 mg kg^{-1}, 2.1 mg C-mic g^{-1} ss, y 15.9 mg CO_2 en 100 g^{-1} s $hora^{-1}$, respectivamente. En todos los suelos predominó la biomasa fúngica, por sobre la bacteriana. Estos resultados demuestran que los suelos del BPM son importantes reservas de C orgánico, sin embargo, actividades antropogénicas generan cambios en la dinámica de la BMS en estos bosques naturales de las estribaciones occidentales de los Andes, provocando alteraciones en el ciclo de los nutrientes. Esta investigación constituye una línea base que ubica al BPM como un punto control para futuros estudios de biogeoquímica regional o global.

Palabras clave: Temperaturas, Modelación, Optimización, Tomate.

1. Introduction

Pre-montane forests, also known as low montane forests in the foothills of the Andes, have large amounts of stored C, forming part of their biomass, both above and below ground (Jobbágy & Jackson, 2000, Stockmann et *al.,* 2013). These mountain forests are characterized by their biodiversity and constitute 26% of the world's forest area (CDS, 2008).

The soils where these pre-montane forests evolved have their genesis from volcanic ash, characterized by low nitrogen (N) concentrations (Huygens *et al.,* 2008), high total phosphorus (P) contents, but with very limited available forms (Redel *et al.,* 2008, Lambers *et al.,* 2012). Under such a scenario of restrictions, these ecosystems have developed compensatory functional strategies, where the transformation and mineralization of SOM and biological nitrogen fixation, among others (Pérez *et al.,* 2010) are key processes, mediated by soil microbial biomass (SMB), which is fundamental in nutrient cycling (Valenzuela *et al.,* 2001). BMS represents only 1-4% of the C and 2-6% of the total N in the soil, its presence is of vital importance, and plays a fundamental role in nutrient cycling (Van der Heijden *et al.,* 2008), however, the size of the biomass reservoir and its microbiota are

influenced by the amount of SOM, climatic factors, physicochemical characteristics, and changes in soil vegetation cover (Dube *et al.,* 2009).

The BMS affects several stages of the biogeochemical cycles (C, N, P and S), and its determination is useful for studies in natural environments such as pristine or little altered ecosystems, under different scenarios of spatio-temporal anthropogenic pressures (Jangid *et al.* 2011). As land use changes are generated by land cover modification, some nutrients are released to the atmosphere, others are stored in the soil, remain on site as dead matter, or are exported by anthropogenic activities or natural processes.

It is known that the forests located in the western foothills of the Andes Mountains are privileged control points for baseline studies of global biogeochemistry, which allow us to answer ecological questions and project potential effects in the face of future scenarios of human disturbance and global climate change, where increasingly prolonged periods of drought affect the productivity and stability of forest ecosystems in the region (Huygens *et al.,* 2011).

Anthropogenic activities imply changes in the natural landscape as a result of forest clearing for agricultural and silvicultural purposes, fragmenting the ecosystems and forming a true mosaic of isolated vegetation covers and multiple land uses, different from the original one.

In this sense, the present research sought to quantify the impact of anthropogenic pressures/activities on soil microbial biomass content, organic C content and $_{CO2}$ emissions in soils of the Murocomba Protected Forest, located in the western foothills of the Andes Mountains.

2. Materials and Methods

The field research was conducted in and around the Murocomba Protected Forest (BPM). The BPM is located in a remote area, at an altitude between 350 - 1500 m.a.s.l., with two distinct climatic seasons. According to Holdridge, it comprises the life zones "Pre-montane very humid forest" and "Pre-montane rainforest". The rainy season contributes 85% - 90%, and the dry season between 10% - 15% of the rainfall. Rainfall varies according to altitude, with an increasing third order polynomial distribution. At 350 meters above sea level, rainfall averages 2000 mm, but when the elevation reaches 900 - 1300 meters above sea level, rainfall can exceed 4500 mm. The average annual temperature is modal, with 23°C (March - April) and 18°C (July - August). The average annual relative humidity depends on the climatic season and ranges between 85 - 87% (rainy season) and 79 - 84% (dry season) (Cuásquer *et al*., 2008).

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The processing and analysis of samples was carried out at the Environmental and Plant Microbiology Laboratory of the State Technical University of Quevedo, located at the Manuel Haz Álvarez Campus, Km 1.5 of the Quevedo-Quito highway.

Treatments and study plots. Five treatments based on vegetation cover (land use) with different levels of anthropogenic intervention (scenarios) were established in remote areas of the BPM. For this purpose, three plots (replicates) of 100 ^{m2} (10 m x 10 m) each, representative of each treatment, were delimited for each treatment. Table 1 details the treatments according to current land use:

Table 1. *Treatments based on vegetation cover (land use) in Murocomba Protected Forest.*

Codes	Treatments
T1	Primary forest (control)
T2	Secondary forest
T3	Regenerating forest
T4	Pasture
T5	Planting of *Gmelina*

Soil sample collection . In the rainy climatic season of 2018, three soil sub-samples were collected from all plots at a depth between 0 - 20 cm, from which a composite sample was constituted for each plot, which was equivalent to three samples/replicates (n=3) per treatment. The soil samples were transferred to the Environmental and Plant Microbiology laboratory of the UTEQ in insulated boxes, where rocks, plant debris, and macro-invertebrates were removed. The fresh soil (without previous drying) was sieved through a 2 mm mesh and stored at 5° C for later analysis. At the same time, the soil samples were subjected to chemical analysis: pH, total organic carbon ($_{Corganic}$), total nitrogen (Nt), C/N ratio, phosphorus (P), potassium (K), and magnesium (Mg) according to the methodology used by (Sadzawka *et al.* , 2006).

Field capacity. Sieved soil samples were oven-dried for 72 h at 60 °C to constant weight. Then, 100 g-1 of dry soil per sample was placed in a 100 mL-1 test tube, the volume occupied by the soil mass was recorded, added 5 mL-1 of water (dropwise) in the center and capped the test tube. After 24 hours, the volume of soil that was not hydrated (dry soil) was recorded (Sadzawka *et al.*, 2006). The calculation of field capacity (%) was performed employing the equation used by Silva *et al*. (2015):

$$\%CC = \frac{V1}{(V1 - V2)} * \frac{5mL^{-1} de\ H_2O}{100g^{-1} de\ suelo} * 100$$

Where:

V1= Initial volume (volume occupied by g-1 of soil)
V2= Final volume (volume that has not been wetted)
CC= Field Capacity

Moisture content. Wet soil samples of known weight were placed in an oven at 60 °C for 72 hours, until a constant weight was obtained. Subsequently, the dry soil was again weighed and the moisture content was calculated using the following equation (Silva *et al*., 2015):

$$\%\ H = \frac{(Ph - Ps)}{Ps - Ph} * 100$$

Where:

Ph=Wet soil **weight**
Ps= Dry soil weight
%H =Percentage of humidity

Amount of water to add to the samples. Once the field capacity and moisture content were determined, the amount of water needed to add to the soil was estimated according to the equation described below (Silva *et al*., 2015):

$$H_2O\ a\ añadir\ (mL^{-1}) = \frac{(CC_{?\%} - \%H)}{\%H + 100} * g^{-1}\ de\ suelo$$

Where:

$CC_{?\%}$ =FIELD CAPACITY TO BE DETERMINED =Field capacity to be determined
%H =Percentage of soil moisture

Soil active microbial biomass (AMB). It was determined by the substrate-induced respiration technique (RIS), described by (Chiu *et al.,* 2006; Ananyeva *et al.,* 2006). For this purpose, 10 g of soil at field moisture, previously sieved, were placed in glass chambers (100 mL capacity) and stabilized for 24 hours at room

temperature. Subsequently, the soil was mixed with 10 mg of glucose (1 mg g-1 of soil), dissolved in the amount of water (sterile distilled) necessary to adjust the samples to 80% of their water retention capacity. CO_2 released during the incubation period (6 h at 22 ^{oC}) was trapped in NaOH solution (0.1 M) and titrated with HCl (0.1 M). The BMA was calculated on the basis that 1 mL of HCl (0.1 M) is equivalent to 2.2 mg CO_2 and that for a respiration coefficient equal to 1: 1 mg $CO_{2/100}$ g h = 20.6 mg C-biomass/100 g.

Selective fungal and bacterial inhibition. Streptomycin and chloramphenicol were used as bacterial inhibitors, while cycloheximide and captan 80 were used as fungal inhibitors. The selection and concentration of the antimicrobials applied to the soil were carried out according to the reports of (West, 1986; Bailey *et al.* , 2002; Nakamoto & Wakahara, 2004). As with glucose, the antimicrobials were mixed with the soil, and sufficient water was applied to moisten the soil, without saturating it. The CO_2 detected represented the response to the inhibition of respiration caused by the microbial inhibitors and was expressed in mg C-mic g-1 of soil. To determine the fungal (BF), bacterial (BB), and residual (BR) biomass in each of the soil use treatments, the triplicate samples received the following combination of antimicrobials (Table 2):

Table 2. *Combination of soil, glucose, and antimicrobialsto determine BF, BB, and BR.*

1.	Soil + glucose (1 mg g-1soil).
2.	Soil + glucose (1 mg g-1) + streptomycin (32 mg g-1) + chloramphenicol (32 mg g-1).
3.	Soil + glucose (1 mg g-1) + cycloheximide (20 mg g-1) + captan (20 mg g-1).
4.	Soil + glucose (1 mg g-1) + streptomycin (32 mg g-1) + chloramphenicol (32 mg g-1) + cycloheximide (20 mg g-1) + captan (20 mg g-1).

BF, BB, and BR were calculated according to West (1986): A = active microbial biomass; (A-B) = fungal biomass; (A-C) = bacterial biomass; D = residual biomass; (A-B)/(A-C) = fungi/bacteria ratio. The percentage inhibition of microbial biomass caused by the use of antibiotics individually and in combination was determined according to the following equations:

$IBB = [(A - C)/A]_{*100}$
$IBF = [(A - B)/A]_{*100}$
$IBR = [(A - D)/A]_{*100}$

Where:

IBB=Percentage of inhibition by combination of antibiotics.
IBF =Percentage of inhibition by combination of antifungals.
IBR =Percentage of inhibition by combination of antibiotics and antifungals.

The following equations were used to estimate the proportion of fungal and bacterial biomass:

$100[\{(A - B) + (A - D)\} / 2]/(A - D)$
$100[\{(A - C) + (B - D)\} / 2]/(A - D)$

Inhibitor additivity ratio (IAR). It was calculated according to Beare *et al.* (1990), using RIS. It was expressed as the microbial biomass of soils treated with antibiotics (streptomycin + chloramphenicol) to inhibit bacteria, antifungals (cycloheximide + captan) to inhibit fungi, and the simultaneous use of inhibitors of both microbial groups. Intact soil (without antimicrobials) was also used. It has been established that when RAI is equal to 1.0, antimicrobials do not exert inhibitory effect on other organisms for which they were not designed. Whereas an additivity ratio >1.0 indicates that antimicrobials have an inhibitory effect on other organisms for which they were not designed. An additivity ratio <1.0 shows that they exert a stimulatory effect on microorganisms (Beare et *al.,* 1990; Nakamoto & Wakahara, 2004). RAI was determined by the following equation.

$RAI = [(A - B) + (A - C)]/(A - D)$

Total inhibition by combined effect of inhibitors (ITC). This variable expresses the percentage of microbial biomass inhibited by the combination of antimicrobials: antibiotics (streptomycin + chloramphenicol) and antifungals (cycloheximide + captan), (Chiu *et al.,* 2006; Susyan *et al.,* 2011). It was calculated based on the equation described below.

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ITC = {(A - D) / (A)}*100

Potential $_{CO2}$ emissions from soil at laboratory level. For this purpose, 10 g of soil at field humidity, previously sieved in a 2 mm mesh opening, were placed in glass chambers (100 mL capacity) and stabilized for 24 hours at room temperature. Subsequently, the soil was mixed with 10 mg of glucose (1 mg g-1 of soil), dissolved in the amount of sterile distilled water necessary to adjust the samples to 80% of their water retention capacity. $_{CO2}$ released during the incubation period (6 h at 22 ^{oC}) was trapped in a NaOH solution (0.1 M) and titrated with HCl (0.1 M).

Statistical analysis. In order to determine the effects of vegetation cover and anthropogenic interventions (treatments) on soil microbial biomass, the data obtained were subjected to an analysis of variance (ANOVA) with a significance level of 95% (*P < 0.05).* Subsequently, the LSD (least significant difference) test was applied, with a significance level of 95% (*P < 0.05). The* statistical package *SYTAT 11* version for Windows was used for the effect.

3. Results

Soil chemical analysis. Significant statistical differences (*P ≤ 0.05*) were detected in the soil chemical analyses (pH, NH4, P, K, MO and $_{Corganic}$) among the treatments under study (soil uses). For pH (*F=16*.35; *P=0.*000) soils subjected to livestock activities (pasture) presented the highest acidity levels with 5.30, placing them in the "strongly acid" category, while soils of the other treatments were in the range of 5.50 to 6.0, which places them in the "moderately acid" category. Regarding cations, it was detected that the highest available NH4 concentrations (*F=8*.53; *P=0.*002) were in the treatments: primary forest soil, secondary forest soil, and *G. arborea* plantation soil, with 19.0, 16.5, and 19.5 ppm, being higher and different from the other treatments. For P (*F=11*.90; *P=0*.000), K (*F=22*.56; *P=0*.000), primary forest, secondary forest and regenerating forest soils presented the highest concentrations, with values of 13.0, 10.5 and 9.5 ppm (P); 0.42, 0.51 and 0.29 (meq/100 mL) (K), respectively, being significantly higher than pasture and *G. arborea* plantation soils.

The MO contents (*F=14*.62; *P=0.*000) were statistically higher in the primary forest soils, with 3.4%, being different from the secondary forest, regenerating forest, *G. arborea* plantation and pasture soils, which showed ranges from 2.7% to 2.15%. Regarding soil $_{Corganic}$ (*F=14*.65; *P=0*.000), the treatments that presented the highest concentrations were the primary forest, regenerating forest and *G.*

arborea plantation soils with 20.0, 15.4 and 15.7 (mg/kg), in contrast to the secondary forest and pasture soils that presented values of 14.0 and 12.5 mg/kg (Table 3).

Table 3. *Chemical variables analyzed in soils with different vegetation cover (anthropogenic uses). Murocomba Protected Forest, Valencia, Ecuador.*

TREATMENTS	Ph	NH4 (ppm)	P (ppm)	K (meq/100 mL)	MO (%)	C (mg/kg)
Primary forest (control)	5.6 ± 0.1 **b**	19.0 ± 2.0 **a**	13.0 ± 2.0 **a**	0.42 ± 0.06 **a**	3.4 ± 0.20 **a**	20.0 **a**
Secondary forest	6.0 ± 0.2 **a**	16.5 ± 6.5 **a**	10.5 ± 0.5 **ab**	0.51 ± 0.10 **a**	2.4 ± 0.10 **bc**	14.0 **bc**
Regenerating forest (fallow)	6.0 ± 0.1 **a**	8.0 ± 2.0 **b**	9.5 ± 2.5 **b**	0.29 ± 0.03 **b**	2.65 ± 0.15 **b**	15.4 **b**
Pasture	5.3 ± 0.0 **c**	9.0 ± 1.0 **b**	6.0 ± 0.0 **c**	0.18 ± 0.02 **c**	2.15 ± 0.25 **c**	12.5 **c**
Gmelina arborea plantation	5.5 ± 0.2 **bc**	19.5 ± 1.5 **a**	6.0 ± 1.0 **c**	0.17 ± 0.01 **c**	2.7 ± 0.30 **b**	15.7 **b**

Values correspond to averages of three replications with their respective standard deviation. Equal letters indicate statistically similar means ($P < 0.05$).

Active microbial biomass and fungal biomass/bacterial biomass ratio. Significant statistical differences ($P < 0.05$) were detected between soils with different vegetation cover (anthropogenic uses), for the variables: active microbial biomass (AMB) (F=7.60, P=0.030), fungal biomass (BF) (F=5.30, $P=0.000$), bacterial biomass (BB) ($F=4.35$, $P=0.000$), fungal biomass/bacterial biomass (BF/BB) ratio ($F=1.15$, $P=0.000$), while for the residual microbial biomass (BMR) variable no differences were found ($F=.6.10$, $P=0.03$). The highest BMA contents were detected in the soils of primary forest (control) and secondary forest, with 6.65 mg C-mic g-1 dry soil (mg C-mic g-1 ss), and 5.75 mg C-mic g-1 ss, respectively, being statistically similar but higher than the contents found in the soils of regenerating forest, and *G. arborea* plantation, with 6.65 mg C-mic g-1 dry soil (mg C-mic g-1 ss), and 5.75 mg C-mic g-1 ss, respectively, being statistically similar but higher than the contents found in the soils of regenerating forest, and plantation of *G. arborea*,

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with 5.40 mg C-mic g-1 ss, and 5.10 mg C-mic g-1 ss. However, the lowest BMA contents were detected in the pasture soil, with 2.10 mg C-mic g-1 ss.

In all treatments, BF predominated over BB, which is reflected in the BF/BB ratio higher than 1.11 for all treatments. The soil from primary forest showed the highest values of BF, BB, and BF/BB with 3.75, 2.27, and 1.65 mg C-mic g-1 g-1 , respectively. While in the soil from the pasture, lower contents were detected. The BMR values found in all soils were statistically similar (Table 4).

Table 4*. Active (AMB), fungal (FB), bacterial (BB), residual (BR) microbial biomass contents in mg g-1 of dry soil, and fungal biomass/bacterial biomass ratio (BF/BB) in soils with different vegetation cover (uses). Murocomba Protected Forest, Valencia, Ecuador.*

TREATMENTS.	**BMA (mg g-**	**BF (mg g-**	**BB (mg g-**		**BMR (mg**
Primary Forest	6.65 (±0.15) a	3.75 (±0.20) a	2.27 (±0.38) a	1.65 (±0.80) a	0.63 ns
Secondary forest	5.75 (±0.27) a	3.26 (±0.09) a	2.15 (±0.55) a	1.51 (±0.92) a	0.34 ns
Regenerating forest	5.40 (±0.35) b	2.95 (±0.41) b	1.72 (±0.18) b	1.71 (±0.36) a	0.73 ns
Pasture	2.10 (±0.25) c	0,80 (±0.33) c	0.72 (±0.20) c	1.11 (±0.22) b	0.58 ns
Plantation of *Gmelina arborea*	5.10 (±0.35) b	2.90 (±0.65) b	1.80 (±0.41) b	1.61 (±0.75) a	0.35 ns

Values correspond to averages of three replications with their respective standard deviation. Equal letters indicate statistically similar means ($P < 0.05$).

Inhibitory effect of antimicrobials . No significant statistical differences ($P < 0.05$) were detected for the variables inhibition of fungal biomass (% IBF), inhibition of bacterial biomass (% IBB), and inhibition by combined effect of antifungals and antibiotics (% ITC), as well as in the additivity ratio of inhibitors (RAI), (Table 5).

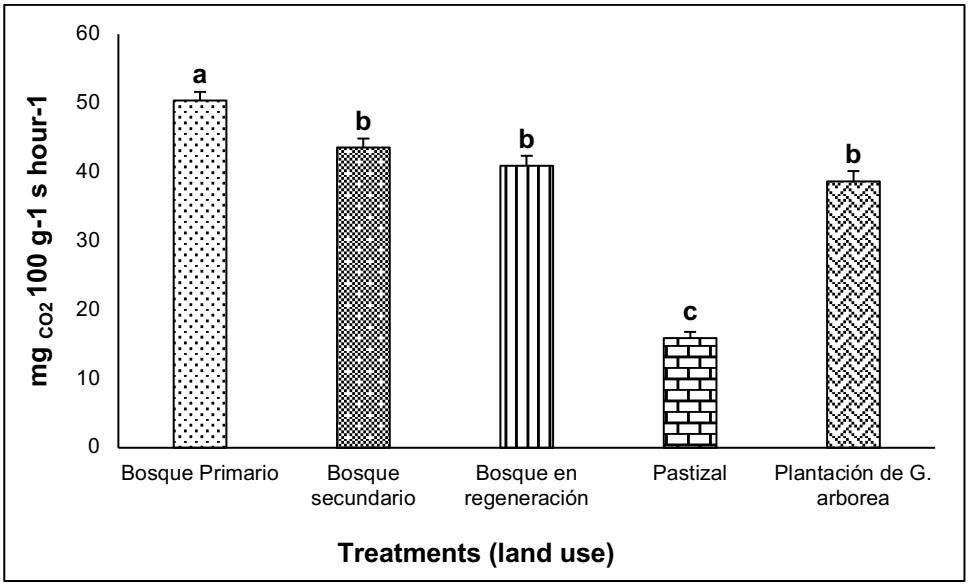
Table 5. *Inhibition percentages of fungal biomass (% IBF), bacterial biomass (% IBB), inhibition by combined effect of antimicrobials (% ITC), and inhibitor additivity ratio (RAI), in soils with different vegetation cover (uses). Murocomba Protected Forest, Valencia, Ecuador.*

TREATMENTS.	**% IBF (C + C) ***	**% IBB (E + C) ***	**% ITC (F + A) ***	**RAI * RAI**
Primary Forest	44.35 (±8.40) ns	29.44 (±11.50)	83.79 (±7.08) ns	1.07 (±0.06) ns
Secondary forest	50.27 (±6.46) ns	27.33 (±18.47)	87.60 (±5.82) ns	1.14 (±0.24) ns
Regenerating forest	47.85 (±7.10) ns	26.99 (±3.81) ns	84.84 (±6.43) ns	1.08 (±0.09) ns
Pasture	46.81 (±12.49) ns	20.86 (±7.80) ns	87.67 (±2.03) ns	1.35 (±0.21) ns
Planting of *Gmelina arborea*	45.98 (±5.60) ns	28.61 (±6.04) ns	84.59 (±3.80) ns	1.18 (±0.12) ns

Values correspond to averages of three replications with their respective standard deviation. Equal letters indicate statistically similar means ($P < 0.05$).

CO_2 emissions from the soil. Significant statistical differences were detected among treatments ($F=4.2$, $P=0.03$). Soils from primary forest released 50.38 mg CO_2 in 100 g-1 soil hour-1 (mg CO_2 100 g-1 s hour-1), which emissions were statistically higher than those from secondary forest, regenerating forest and *G. arborea* plantation soils, with 43.56, 40.91 and 38.64 mg CO_2 100 g-1 s hour-1, respectively. While Grassland soils released 15.91 mg CO_2 100 g-1 s hour-1, emissions statistically lower than those released from soils with other vegetation covers (Figure 1).

Figure 1. CO2 emissions (mg CO_2 100 g-1 s hour-1) at laboratory level, from soils with different vegetation cover (uses). Murocomba Protected Forest, Valencia, Ecuador.



Values correspond to averages of three replications with their respective standard deviation. Equal letters indicate statistically similar means ($P < 0.05$).

The higher soil $_{corganic}$ contents detected in the treatments with forest cover: primary forest, secondary forest, regenerating forest and *G. arborea* plantation (20.0, 14.0, 15.4 and 15.7 mg/kg, respectively), would probably be associated with the presence of available SOM in the soils, given by constant contributions of fine and coarse litter, and the progressive decomposition of the same, unlike the Pasture soils where lower contents of $_{Corganic}$ were obtained, This is attributable to the decrease in the availability of C and N in the SOM, as a consequence of its accelerated mineralization, changes in the microclimate, volatilization into the atmosphere and nutrient leaching mechanisms, factors associated with intensive livestock activity (Galicia *et al.,* 2016; Céspedes-Flores *et al.,* 2018). This phenomenon has been detected in other studies of forest-covered soils, where internal recycling, conservation mechanisms and nutrient retention are efficient in ecosystems similar to the forest-covered soils analyzed in the present study (Zanabria & Cuellar, 2015; Suárez-Duque et *al.,* 2016), with higher edaphic microbial biomass concentrations than those ecosystems intensely anthropogenically intervened, such as pasture soils.

In all treatments, BF predominated over BB, which is reflected in the BF/BB ratio higher than (1.11). The primary forest treatment showed the highest values of BF, BB, and BF/BB with 3.75 mg C-mic g-1 ss, 2.27 mg C-mic g-1 ss, and 1.65, respectively, due to the fact that the abundance of bacteria per unit of organic matter was less variable than fungal biomass, which correlates with the findings

reported by Findlay *et al.* (2002), who indicate that bacteria are a very predictable component within the BMS; however, bacterial populations, despite having more individuals (cells) per unit weight than fungal populations, are smaller, but no less important for the biogeochemistry of ecosystems. The low BF contents in grassland soil would be due to the very low organic matter input and availability for BMS, compared to forest-covered soils.

The highest $_{CO2}$ emissions from the primary forest floor (50.38 mg $_{CO2}$ in 100 g-1 ss hour-1), are surely associated with the intense activity of the BMS during the process of biodegradation and mineralization of supplies of fine litter (leaf litter, fine branches, flowers, fruits, bark) and coarse litter (trunks, thick branches, roots, fallen tree stumps) abundant in this type of ecosystems, compared to a lower contribution of biomass to the soil by other vegetation covers such as Grasslands (15.91 mg $_{CO2}$ in 100 g-1 ss hour-1), which are associated a lower BMS activity due to a lack of carbonaceous resources, as pointed out by Céspedes-Flores *et al.* (2018). $_{CO2}$ emissions from secondary forest, regenerating forest and *G. arborea* plantation soils (43.56, 40.91, 38.64 mg $_{CO2}$ in 100 g-1 ss hour-1, respectively) show that their organic matter inputs and BMS size are similar, which would indicate that $_{CO2}$ release from these types of ecosystems is sustained and conserves soil C stocks. On the other hand, the fact that the forest-covered soils analyzed in this research release more $_{CO2}$ than grasslands does not mean that transforming these ecosystems into grasslands would prevent the release of C from the soil; on the contrary, most of the conserved C would be released. While carbon cycling in forested ecosystems is very dynamic, with higher C inputs that are immobilized for long periods of time (residence time) in the plant biomass, with a gradual and lower release of $_{CO2}$ compared to that fixed by the ecosystem. In this sense, the scientific literature shows that the BMS pool and its metabolic activity is closely related to the contributions of carbonaceous materials, results of the net primary production within terrestrial ecosystems (Pardo-Plaza *et al.,* 2019; Rosero *et al.,* 2019), a situation that correlates with the results obtained in this research.

5. Conclusions

The soils of the "Bosque Protector Murocomba" are subject to anthropogenic pressures, whose changes in use imply modifications in the soil microbial biomass, nutrient balance, and $_{Corganic}$. Forest cover contributes to the conservation of $_{Corganic}$ stored in the soils of this protective forest, being the conversion of these soils to pasture the main cause of C loss from the soil pool. Soil microbial biomass can be used as a sensitive and robust bioindicator of disturbance or early

anthropogenic changes in the soils of the BPM. This research is the first report of the use of soil microbial biomass as a biological indicator of anthropogenic changes in soils of this type of ecosystems, already scarce and very sensitive, located in the western foothills of the Ecuadorian Andes, and constitute a baseline for future studies of local, regional and global biogeochemistry.

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