SOIL FERTILITY IMPROVEMENT BY LITTER DECOMPOSITION AND INOCULATION WITH A SOIL FUNGUS IN AVOCADO PLANTATIONS OF COLOMBIA

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ABSTRACT

The aim of this study was to evaluate the effect of litter decomposition and its inoculation with the fungus *Mortierella* sp. on soil fertility in three Andisols cultivated with avocado cv. Hass. For this purpose, litterbags containing avocado litter were either uninoculated or inoculated and distributed around the trees. As control litter was not allowed to accumulate on the soil surface. Eighteen months later soil surface samples were collected just below the litterbags and control sites. Soil pH, K, Ca, P, Mg, and B contents significantly increased with the presence of litter and in some sites the increase was higher with inoculation. By contrast, soil Al, Fe, and Mn were significantly lower, whereas Zn was higher when litter was present compared to control. The results showed that some soil fertility parameters evaluated were enhanced when litter was present and even more when the litter was inoculated with the soil fungus.

Key words: *Persea americana*, *Mortierella* sp., nutrient cycling, Andisols.

INTRODUCTION

One of the least studied aspects of tropical soils, and particularly of those planted with fruit crops, is the effect of leaf litter (LL) decomposition can have on soil fertility (Wang et al.2008). The quality of the LL can determine microbial activity and nutrient release (McGrath *et al*. 2000, Kumar and Agrawal 2001, Villela and Proctor 2002, Singh et al. 2004, Weerakkody and Parkinson 2006, Liao et al. 2006).

This is very important in the tropics due to the fact that most soils are very acidic (pH <5.5), rich in exchangeable Al, and poor in plant essential nutrients (Zapata 2004), which restricts plant production. In order to compensate for these problems it is necessary to apply high quantities of lime and fertilizers, which increases production costs (Osorio 2014). It is thought that the production and microbial decomposition of leaf litter could improve soil quality (Huang et al. 2007; Martinez et al. 2014) and thus help fix the problems associated with these acidic soils. However, few reports exist on tropical soils to validate this assessment. It is expected that this process can make nutrients newly available to plants, thus improving soil quality and contributing to the soil structure and water and nutrient retention capacity (Rodríguez et al. 2011). In this way, nutrient cycling would permit long-term

agroecosystem sustainability (Gelvez 2008; Arias 2001; Sierra et al. 2009 ;Castellanos and León 2011). One example of this situation occurs in avocado plantations (*Persea americana* Mill.). This crop is highly adaptable and its production in 2014 was 3.9 million tons, more than 86% of which occurred in tropical developing countries (FAO 2013). In Colombia, interest has been growing in the Hass cultivar, for both national and international markets (Bernal and Díaz 2014; DANE 2013). However, its productivity, as mentioned previously, can be severely limited by low soil fertility (Tamayo and Osorio 2014). Our hypothesis in this study is that microbial decomposition of avocado cv. Hass leaf litter can improve soil fertility by increasing nutrient availability and correcting soil acidity. For this reason, a study was established to determine the effect of leaf litter decomposition of avocado cv. Hass, either uninoculated or inoculated with the *Mortierella* sp. fungus on soil fertility parameters in three plantations of contrasting altitude in Colombia.

MATERIALS AND METHODS

Experimental sites

The study was conducted in three five-year-old avocado cv. Hass plantations. The trees were grafted on Antillean rootstocks grown in three production zones in the department of Antioquia, Colombia (Támesis, Jericó and Entrerríos) (Table 1). The distance between trees was 5x7 m (285 trees ha^{-1}). SpecWare 9 Pro®, Spectrum Technologies Inc. (Version 9.03 Build 0240) automated meteorological stations were established in these localities (Table 1). In order to characterize the soil fertility in each site, a composite soil sample was taken at random in the area around 15 trees. Soil tests were performed in the Soil Laboratory of the Universidad Nacional de Colombia at Medellín (Table 2).

Leaf litter

Senescent leaves (leaves and their petioles) were collected directly from the trees in each location. The leaves were oven dried at 65°C until reaching constant weight. They were then packed in 20x20-cm litterbags made of plastic fiber mesh (pore sizes 1x1 mm). Each bag was filled with 10 g of leaf litter (dry based). The bags were sewn closed with nylon thread, placed on the ground and secured with staples, in order to ensure that they would not be taken by rodents or lost due to surface runoff. The litterbags were distributed randomly in the field around trees.

Table 1.Description of the sites where leaf litter decomposition studies were conducted.

Site	Altitude (m)	Temperature (ºC)	Rainfall (mm)	Air humidity (%)	Sunshine (h)	Ecological Life zone*	USDA soil taxonomy**
Támesis	1350	23	1900	85	1726	PMF	Ultic Melanudand
Jericó	1900	18	2500	85	2430	LMMF	Typic Hapludand
Entrerríos	2420	16	1900	83	1684	LMMF	Ultic Melanudand

*Espinal and Montenegro (1977).

**Soil profiles were described and classified by Professor Juan Carlos Loaiza, Universidad Nacional de Colombia.

Table 2.Soils chemical characteristics in the three sites (Támesis, Jericó, and Entrerríos)**.**

Site	pH	SOM	Al	Ca	Mg	K	Na	P	S	Fe	Cu	Mn	Zn	B
		%	----------$cmol_c\ kg^{-1}$----------					----------mg kg^{-1}----------						
Támesis	4.8	28.4	2.4	1.2	0.6	0.25	0.05	8	7	58	2	5	20	0.2
Jericó	5.0	13.2	1.7	2.4	0.9	0.37	0.01	13	43	115	2	6	4	0.1
Entrerríos	5.4	25.8	1.6	3.4	4.8	0.76	0.16	20	43	177	16	49	7	2.1

Methods: soil pH in water (1:1); soil organic matter (SOM) by Walkley and Black; Al extracted by 1 *M* KCl; Ca, Mg, K, and Na extracted by 1 *M* ammonium acetate; P extracted by Bray II; S extracted by 0.008 *M* calcium phosphate; Fe, Mn, Cu, and Zn extracted by Olsen-EDTA; B extracted by hot water

Inoculum

The fungus *Mortierella* sp. (Dyal and Narine 2005) was used as a fungal inoculum. The fungus was obtained from the Ecology and Environmental Conservation Laboratory, and was originally isolated from an Andisol in Hawaii, USA by Osorio and Habte (2001). Since then, the fungus has been multiplied aseptically in YMA medium (Yeast Mannitol Agar) and preserved under refrigeration at 4°C for experimental use. For the present study, the fungus was multiplied in YMA medium for five days and the spores and mycelium were then aseptically suspended in sterile distilled water in order to obtain a concentration of $1x10^7$ CFU/mL.

Treatments

The treatments consisted of depositing decomposition bags containing leaf litter that was either uninoculated or inoculated with the fungal suspension containing the *Mortierella* sp. For this purpose 10 g of avocado leaf litter (dry based) were sprayed with 2.5 mL of the fungal suspension and later transferred into litterbags. Uninoculated leaf litter was sprayed with 2.5 mL of sterile distilled water per bag. The bags (inoculated and uninoculated) were immediately transferred to each of the plantations and distributed at random within the protected root zone, as explained previously. As control, in some trees leaf litter was not allowed to accumulate on the soil surface.

Experimental design

Each site (Jericó, Támesis and Entrerríos) was independently studied. In each location, a completely randomized design was employed; the treatments had a 3x3 factorial arrangement, which corresponded to soil without leaf litter (control), soil with uninoculated leaf litter (LL), and soil with inoculated leaf litter (ILL). Soil sampling was conducted just below (0-5 cm) the litter bags (LL or ILL) or in the control sites at 18 months after the treatments were deposited. Each sampling had four repetitions.

Variables

The soil samples collected were sent to the Soil Chemistry and Plant Tissue Laboratory of CORPOICA-Tibaitatá (Mosquera, Cundinamarca, Colombia). The following variables were

determined: soil organic matter (SOM) content (Walkley and Black); extractable P (Bray II); Ca, Mg and K (1 *M* ammonium acetate, pH 7.0); Al (1 *M* KCl); effective cation exchange capacity (ECEC) sum of Al, Ca, Mg and K; Fe, Mn, Zn, Cu (modified Olsen) and B (monobasic calcium phosphate). The protocols followed are described in Westermann (1990).

In order to confirm the presence of the *Mortierella* sp. fungus in the samples with inoculated leaf litter (and its absence in the uninoculated samples), 1 g aliquots of the decomposed leaf litter samples were suspended in 9 mL of sterile distilled water (10^{-1} dilution). Serial dilutions were prepared up to 10^{-3}; 100 µL was taken from the 10^{-2} and 10^{-3} dilutions, and was aseptically transferred to Petri dishes containing a selective medium for the fungus, developed by Osorio (2008). This contained YMA medium with streptomycin sulfate (500 mg L^{-1}), tetracycline (0.1 mg L^{-1}), benomyl (75 mg L^{-1}), and cyclohexamide (100 mg L^{-1}). The Petri dishes were incubated for 48 h at 28°C. After this incubation period, the fungal colony forming units (CFU) were counted. Samples were also taken of the surface soil in uninoculated sites, in order to confirm the absence of the fungus.

Data analysis

The data were subjected to analysis of variance (F test) and to the Duncan's multiple range test for mean separation (t-test). In both cases, the level of significance (P) used was ≤ 0.05. The analyses were carried out using Statgraphics Centurion software, version 16.

RESULTS

Soil chemical properties

Statistically significant differences in soil pH were found in the Támesis site ($P \leq 0.0185$); the value was higher for the treatments that included uninoculated LL and ILL. For the Jericó site, there were highly significant differences ($P \leq 0.001$) among the treatments when the leaf litter was inoculated with *Mortierella* sp. A similar situation occurred in Entrerríos ($P \leq 0.001$), where the soil pH also improved when ILL was present (Table 3). In Entrerríos, significant differences were found ($P \leq 0.05$) among the treatments; in that site, the SOM content decreased when inoculated LL was applied. In the other sites (Támesis and Jericó), the SOM content was similar for all of the treatments, and no effect was found for the application of LL alone or inoculated with *Mortierella* sp. (Table 3).

In all three sites highly significant differences were observed ($P \leq 0.01$) among the treatments regarding the value of Bray II-P, but the effect was different in each site. For instance, in Támesis control sites had 28.7 mg kg^{-1}, but when the LL was present the value was significantly higher (4.2 times) and this effect was even significantly higher when LL was inoculated with *Mortierella* sp. (ILL) (6.4 times). In Entrerrios, control sites had a value of Bray II-P of 15.6 mg kg^{-1}, the presence of LL did not increased significantly this value, but the ILL did it significantly (43.7 mg kg^{-1}), which represented an increase of 2.8 times. By contrast, in Jericó only there was a significant increase (4.5 times more than in control sites) with LL, but not with ILL (Table 3).

In regard to the effective cation exchange capacity (ECEC), the treatments and the sampling times were both found to have a significant effect in the Támesis site. The ECEC increased when LL was applied alone or inoculated with the decomposer fungus. Significant differences ($P \leq 0.0038$) were only found for the treatments in Jericó. In Entrerríos, the ECEC increased when inoculated LL was applied, presenting differences from the control treatment and uninoculated LL application (Table 3).

The values of exchangeable K were significant differences due to treatments in Támesis and Jericó; but this situation did not occur in Entrerríos. In Tamesis, there was a significant increase in the exchangeable K concentration respect to the control when the leaf litter was inoculated with *Mortierella* sp (ILL) (2.4 times more); in Jerico the significant increase was due to the presence of LL, without effect of inoculation of LL, the increase if exchangeable K was 1.6 times (Table 3). Highly significant differences ($P \leq 0.01$) were found regarding the exchangeable Ca among the treatments in all of the sites. As in the prior case, there was an increase in the Ca concentration for the treatments that had LL and LL inoculated with *Mortierella* sp. In Entrerríos, the increases in Ca were only significant when the LL was inoculated with *Mortierella* sp. (Table 3). Significant differences were also found among the treatments regarding the exchangeable Mg; this increased when the LL was inoculated with the *Mortierella* sp. decomposer fungus. This situation occurred in Támesis, Entrerríos and Jericó, where the exchangeable Mg was higher and it was statistically different (Table 3).
The value of exchangeable Al was significantly affected in all sites with the treatments. For instance, in Támesis in the control the value of Al was 1.6 $cmol_c\ kg^{-1}$ and when the LL was present the value was reduced by 50%, with the treat ILL the reduction was not significant. In Jericó the control had 2.6 $cmol_c\ kg^{-1}$ and with LL and ILL the value was reduced by 2-3 times less. In Entrerrios the behavior was similar, the control had 2.3 $cmol_c\ kg^{-1}$ and with the treatments LL and ILL the value was significantly reduced to 0.53 and 0.03 $cmol_c\ kg^{-1}$, respectively (Table 3).

Table 3 .Soil fertility parameters in avocado cv. Hass plantations in three sites in Colombia subjected to three treatments: soil without leaflitter (control), soil with uninoculated-leaflitter (LL), and soil with inoculated-leaf litter (ILL). Means followed by different letters for a given variable (comparisons in each site) indicate significant differences among the data according to the Duncan's multiple range test ($P \leq 0.05$).

Site	pH (1:1)	SOM	Bray II-P	ECEC	K	Ca	Mg	Al
		(%)	(mg kg-1)	---------- $cmol_c\ kg^{-1}$----------				
Támesis								
Control	4.4 ab	18.3 a	28.7 c	6.1 b	0.36 b	2.8 b	1.0 b	1.6 a
LL	4.7 a	18.6 a	119.8 b	8.9 a	0.44 b	6.0 a	1.7 a	0.8 b
ILL	4.5 b	18.6 a	183.0 a	10.1 a	0.85 a	5.3 a	1.6 a	1.1 ab

Jericó								
Control	4.1 b	35.9 a	22.2 b	0.7 b	0.48 b	2.6 b	1.1 b	2.6 a
LL	4.7 a	34.6 a	101.2 a	11.6 a	0.76 a	7.0 a	2.7 a	0.7 b
ILL	4.6 a	35.1 a	40.5 b	11.4 a	0.76 a	6.6 a	2.5 a	1.2 b
Entrerríos								
Control	4.5 c	30.5 a	15.6 b	11.2 b	0.66 a	5.2 b	2.7 b	2.30 a
LL	5.1 b	23.2 b	19.8 b	9.4 b	0.66 a	5.2 b	2.8 b	0.53 b
ILL	5.9 a	20.8 b	43.75 a	20.2 a	0.81 a	12.0 a	6.3 a	0.03 b

Micronutrients

In all three sites significant differences were found among the treatments regarding the soil boron; In Támesis and Entrerrios there were significant increase (by 3 and 2 times, respectively) in B when the leaf litter was inoculated with *Mortierella* sp., but this did not occur when the LL was uninoculated (LL). On the other hand, in Jerico, the soil B significantly increase (by 1.5 times) with the presence of LL, but the inoculation of LL with the fungus did not improve the value already obtained with the LL alone (Table 4). In the case of soil Zn, there were significant effects with the treatments. In Támesis and Jericó the increase with LL and ILL treatments over the control ranged 3.2-3.3 and 3.5-4.4 times more, respectively. In Entrerrios, the increase was only detected with the LL treatment, but it did not happen with ILL (Table 4).

Regarding soil Fe, there were also significant effects with the treatments, but they were different in each site. For instance, in Tamesis there were not significant effects with the treatments, while in Jerico the presence of LL and ILL reduced the value of soil Fe by about 3 times. In Entrerrios, the increase was only detected with the LL treatment, but it did not happen with ILL. In the case of soil Mn, the trend was significantly to decrease the value of this element when the LL and ILL were present. In Tamesis, the reduction was about 36-43% respect to the control, in Jerico was 36-60% and in Entrerrios the effect was significant with the ILL treatment (by 80%), but it did not occur with the LL alone (Table 4). On the other hand, there were not significant effects of treatments in soil Cu in any of the sites studied

Table 4 Soil micronutrient concentration in avocado cv. Hass plantations in three sites in Colombia subjected to three treatments: soil without leaf litter (control), soil with uninoculated-leaflitter (LL), and soil with inoculatedleaflitter (ILL). Means followed by different letters for a given variable (comparisons in each site) indicate significant differences among the data according to the Duncan's multiple range test ($P \leq 0.05$).

Site	B	Cu	Fe	Mn	Zn
	---------- (mg kg^{-1}) ----------				
Támesis					
Control	0.2 b	5.9 a	158.8 a	26.2 a	10.1 b
LL	0.3 b	7.1 a	149.4 a	16.8 b	33.2 a
ILL	0.6 a	9.3 a	223.8 a	15.0 b	32.3 a
Jericó					
Control	0.2 b	3.1 a	133.3 a	18.3 a	32.8 b
LL	0.3 a	3.6 a	42.3 b	7.3 b	144.1 a
ILL	0.3 a	3.3 a	52.8 b	12.1 b	114.1 a
Entrerríos					
Control	0.5 b	2.8 a	35.2 b	11.8 a	12.0 b
LL	0.6 b	2.7 a	143.2 a	6.8 ab	24.0 a
ILL	1.0 a	2.1 a	50.8 b	2.4 b	12.2 b

Presence of *Mortierella* sp.

At the end of the study, the presence of the *Mortierella* sp. was observed in the in inoculated leaf litter in all three sites (Table 5). The values fluctuated between 1.6 and 2.8 $x10^3$ CFU per g of inoculated leaf litter (ILL). In contrast, no fungal colonies were detected in the uninoculated leaf litter (LL). The fungus was not found in the soil samples in any of the locations when this was not inoculated; this served to confirm that the fungus did not naturally occur in the soil.

Table 5 Number of colony forming units of the fungus *Mortierella* sp. in uninoculated decomposed leaflitter (LL) and leaflitter inoculated with the fungus (ILL) in avocado cv. Hass plantations in three sites in Colombia, 430 days post-inoculation. Means followed by different letters indicate significant differences among the data according to Duncan's multiple range test ($P \leq 0.05$). Horizontal comparisons by site.

Site	Control	LL	ILL
		CFU g^{-1}	
Támesis	0 b	0 b	$2.0x10^3$ a
Jericó	0 b	0 b	$1.6x10^3$ a
Entrerríos	0 b	0 b	$2.8x10^3$ a

DISCUSSION

The results obtained in this study showed that the soil fertility parameters evaluated changed as a function of the application of either LL or ILL with the fungus. Although it is not clear what is the mechanism involved in the pH increase, it seems to be associate with the release of organic acids (e.g., citric and oxalic acids) by the LL decomposition (Osorio 2014). These organic acids and their conjugated bases can form complexes (Al-citrate, Al-oxalate) releasing thus OH^- previously associated with Al ions $Al(OH)^{2+}$, $Al(OH)_2^+$ (Hue et al 1986). In the case of *Mortierella* sp., it has been reported its production of oxalic acid and its tolerance to Al^{3+}. This result is relevant since most soils cultivated in the Andean mountains are strongly acid and rich in exchangeable Al^{3+} ions (Jaramillo 2014; Osorio et al. 2012; and Serna et al. 2012) in avocado orchards in eastern and northern Antioquia.

In the same way, it is quite relevant the increase in soil available P with LL and ILL. In the LL decomposition P was released and thus it increased its availability. The release of P in the ILL was higher, which may be the result of the phosphatase activity of the fungus (Alvarez 2012). Also, the reduction of exchangeable Al can help to increase soil available P. These results are significant because soil P availability is a critical for avocado productivity, particularly in Andisols, which are capable of fixing P ions onto the soil mineral surfaces (Tamayo and Osorio 2014). In all three soils, the absence of leaf litter was associated with low levels of Bray II-P (< 30 mg kg^{-1}), in contrast in those soils with LL and even more with LL inoculated the levels of P was adequate for avocado. Likely, the fungus could dissolve inorganic P in the soil since it is known phosphate solubilizing microorganisms (Vassilev and Vassileva 2003; Jayasinghearachchi and Seneviratne 2005; Relwani et al., 2008; Qin et al. 2009; Singh and Reddy 2011; Khan et al. 2007).

It is clear from the results that the exchangeable bases (Ca, Mg and K) were affected by the treatments. The decomposition process allow the release of these nutrients (Villela and Proctor 20024; Parker 1983; Ngoran et al. 2006; Tamayo et al. 1999; Tamayo and Hincapié 1999 and Tamayo and Hincapié 2007). In some cases the increase was produced by the presence of uninoculated LL and in some cases was even higher when the LL was inoculated with Mortierella sp. (ILL). This may be associated with the reduction of exchangeable Al since Ca, Mg, and K can remove Al from adsorption sites on the surface of soil clays and

oxides Tamayo and Hincapié, 1999. From a practical point of view, the Tamesis and Jerico soils without leaf litter (control) exhibited low levels of Ca (< 5 cmolc kg-1), Mg (< 1.5 cmolc kg-1), and K (< 0.5 cmolc kg-1), while in those soils with either LL or ILL the levels of these nutrients were higher and can be considered adequate for avocado productivity (Tamayo and Osorio 2014). Similar increase in nutrients were reported by other authors in soil cultivated with avocado and other crops with the addition of organic amendment and litter residues (Gelvez 2008; Tamayo and Hincapié 1999; CIAT 1996 and Bubb et al. 1998). Contrasting results were reported in five-year-old *Jacaranda copaia* and *Vochysia guatemalensis* plantations, in which some nutrients decreased overtime (soil P: 6.7 and 5.0 mg L^{-1}; K: 0.13 and 0.10 $cmol_c$ L^{-1}; Ca: 1.2 and 0.90 $cmol_c$ L^{-1}) (Montagnini (2008); Kumar 2008 and Berg 2000.)

Similar effects were detected in micronutrients, for instance in B the soils without leaf litter had low levels (<0.5 mg kg^{-1}), but in those with either LL or ILL the levels were higher, which is result of the LL decomposition, which was accelerated by the inoculation with the fungus (Sundaramoorthy et al. 2010). This has a special relevance because B is usually a critical factor for avocado productivity; B deficiency in avocado produces flower and fruit abscission (Tamayo and Osorio 2014). In the case of Zn the levels in control soils were adequate (5-10 mg kg^{-1}) and with the presence of leaf litter either inoculated or inoculated the soil Zn levels were several times higher. On the contrary, soil Fe and Mn levels were lower with LL and ILL in comparison with control soils, which is consistent with the increase in soil pH because since both type of ions ($Fe^{2+,\ 3+}$ and $Mn^{2+,4+}$) are precipitated with OH^- ions (Zapata 2014).

The presence of the *Mortierella* sp. at the end of this study fungus is relevant because it show its persistence overtime and its ability to adapt to these soils and crops. Microorganisms are the primary source of enzymes, which play a fundamental role in the maintenance and dynamics of nutrients cycling via organic matter decay (Tamayo and Osorio 2014).

Conclusions

The application of leaf litter inoculated with the *Mortierrella* sp. fungus improved the chemical properties of the soil over time in the sites evaluated. In Támesis and Jericó, increases were observed in the K, Ca, Mg and P contents in the soil when LL inoculated with the *Mortierella* sp. fungus was applied. The contents of B (Támesis and Entrerríos) increased their concentration in the soil when applied LL inoculated with the fungus *Mortierella* sp. Zinc content increased their concentration in the soil when applied alone LL and / or inoculated with the fungus *Mortierella* sp.

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