POST-EFFECT OF MYCORRHIZATION ON SOME INDICATORS OF PHYSICAL PROPERTIES FROM A RED FERRALITIC SOIL

M. C. Riera✉, Mayelín Méndez and N. Medina

ABSTRACT. A study was conducted in the experimental area of INCA, Havana province, on a compacted Red Ferralitic soil. A split plot design with four replications was used in order to evaluate four biofertilization systems in two cropping sequences: soybean (*Glycine max* L.)-corn (*Zea mays*)-sweet potato (*Ipomea batata* L) and soybean-sunflower (*Helianthus annus*)-sorghum (*Sorghum vulgaris*). Seeding started in 1998 spring with 32 plots of 100 m^2 (10x10 m). Biofertilizer was applied by seed pelletization before sowing. Soybean was inoculated with *Bradyrhizobium japonicum* (ICA 8001 strain) and *Glomus clarum*, whereas the remaining crops of each sequence were inoculated with *Glomus clarum* and *Burkholderia cepacia*. For each crop, yield, mycorrhizal colonization and endophyte weight percentages were evaluated, while soil evaluations were performed on real density (Dr), bulk density (Da), texture, degree of aggregation, stability coefficient and dispersion index. Results showed a notable influence of biofertilization system and both crop sequences on the degree of aggregation and some indicators of soil physical properties evaluated.

RESUMEN. Se desarrolló un estudio en el área de experimentación del Instituto Nacional de Ciencias Agrícolas (INCA), provincia La Habana, sobre un suelo Ferralítico Rojo compactado, utilizando un diseño de parcelas divididas con cuatro repeticiones, para evaluar cuatro frecuencias de inoculación de HMA en dos secuencias de cultivos: soya (*Glycine max.* L)-maíz (*Zea mays)*–boniato (*Ipomea batata* L.) y soya-girasol (*Helianthus annus*)-sorgo (*Sorghum vulgaris*). La siembra se inició en la primavera de 1998 con 32 parcelas de 100 m^2 (10 x 10 m), utilizando un área de cálculo de 56 m^2. La aplicación de los biofertilizantes se realizó mediante el recubrimiento de las semillas antes de la siembra, siendo inoculada la soya con *Bradyrhizobium japonicum* (cepa ICA 8001) y *Glomus clarum*, mientras los restantes cultivos de cada secuencia se inocularon con *Glomus clarum* y *Burkholderia cepacia*. Para cada cultivo se realizaron evaluaciones del rendimiento, porcentaje de colonización micorrízica y la masa del endófito, mientras en el suelo se evaluaron la densidad real (Dr), densidad aparente (Da), textura, los micro y macroagregados, el coeficiente de estabilidad y el índice de dispersión. Los resultados mostraron una influencia marcada de las frecuencias de inoculación y de las secuencias de cultivos empleados sobre los macroagregados y otros indicadores del estado de las propiedades físicas del suelo.

Key words: biofertilizers, arbuscular mycorrhiza, soil structural units, sequential cropping

Palabras clave: biofertilizantes, micorriza arbuscular, unidades estructurales de suelo, cultivo secuencial

INTRODUCTION

Many agricultural practices help to protect soil and water quality, keeping or increasing its productivity. Such practices have a significant potential for reducing damages in fragile areas and on natural habitat, improving biodiversity indirectly through the mitigation of many effects caused by agrochemical products and the excessive erosion in modern agriculture (1). Practices for improving soil structure, such as gramineae-legumes rotation, also improve aeration and increase moisture-holding, considering incorporation of vegetable residues an important factor in this process.

Arbuscular mycorrhizal fungi (AMF) are effective agents in soil aggregation; therefore, managing them could be seen as a method for improving soil structure under several conditions (2). The ability of such fungi for absorbing water from small pores of soil matrix contributes to the stabilization of aggregates, joining particles by tying them up in the mycelial net, as well as through bending mechanisms, having a marked effect on soil productivity. AMF hyphae present a marked direct effect on the percentage of stable water aggregates (3), as well as on the formation and stabilization of soil aggregates, reduce nutrient leaching and increase the possibilities for plants to supply their future needs for nutrients after mineralization.

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Soil response to microorganisms could be related or not to plant response. Treatments with AMF alone or combined with other microorganisms could constitute a net benefit, in terms of balance of agricultural production and soil preservation in different agroecosystems. Therefore, it is necessary to include soil response in the evaluation of AMF efficiency (4).

An efficient application of AMF and rhizobacteria in the future management of agriculture will depend mainly on the ability for identifying specific functions of those microorganisms within each particular agroecosystem, as well as for including such discoveries in the management strategy (2).

In experiments using pots, some authors (5) found that AMF mycelia alone are able to produce a significant effect on aggregation but, when they are joined to roots, a synergical effect is found. On the other hand, others (6) pointed out that hyphae within soil matrix create the structural skeleton that keeps soil particles joined. However, there are few studies on the influence of AMF on soil physical properties, in tropical areas, under field conditions.

For these reasons, a study was carried out for evaluating the influence of different frequencies of AMF applications, combined with other biofertilizing microorganisms, on some indexes of soil physical properties using different cropping sequences.

MATERIALS AND METHODS

The experiment was developed in the experimental area of the National Institute of Agricultural Sciences (INCA), located in Havana province, on a compacted Red Ferralitic soil. Its main features appear in Table I.

A split plot design with four replications was used for evaluating four biofertilization systems (subplots) in two cropping sequences (main plots), including the following crops: soybean (*Glycine max*)-corn (*Zea mays*)-sweet potato (*Ipomoea batata L.*) and soybean-sunflower (*Helianthus annus*)-sorghum (*Sorghum vulgaris*). Sowing started in 1998 spring with 32 plots of 100 m^2 (10 x 10m), using an estimation area of 53 m^2. Soybean was the first crop used in both sequences and, in each of them, (B1) check was the fertilized variant with 200 kg $N.ha^{-1}$ as urea, whereas 30 kg $N.ha^{-1}$ dose, also as urea, was applied in the remaining treatments. Neither phosphorus nor potassium was applied, since such nutrient contents were high in the soil and easy to assimilate (Table I). Soybean was coinoculated with *Bradyrhizobium japonicum* (ICA 8001 strain) and *Glomus clarum*, by means of seed coating, whereas the remaining treatments of each sequence were coinoculated with *Glomus clarum* and *Burkholderia cepacia*, according to the following biofertilization system:

B1- Without inoculation and 100% nitrogen fertilizer in all crops (check)

B2- Biofertilization (coinoculation) only in the first crop of the sequence and 60 % nitrogen fertilizer

B3- Biofertilization (coinoculation) in the first and second crop and 60 % nitrogen fertilizer

B4- Biofertilization (coinoculation) in all crops and 60 % nitrogen fertilizer.

Yield, percentages of mycorrhizal colonization as well as endophyte weight were evaluated in each crop. Regarding soil, the following indicators were evaluated according to Kaurichev (7): real density (Dr) through pycnometer method, bulk density (Da) by means of cylinder method, analysis of aggregates according to Savvinov's method and using a sieve set following dry via, and analysis of microaggregates by means of Kachinski's method, from 0.25 to 0.01 mm.

The degree of dispersion and structure stability were established by comparing results of aggregates and microaggregates, by means of estimating dispersion coefficient (KD) and stability index (Ie) using the following formulae:

$$\mathbf{KD} = \frac{\mathbf{\%\,arc\,Microstructure}}{\mathbf{\%arc.texture}}\,\mathbf{x\,100}$$

$$\mathbf{Ie} = \frac{\sum \mathbf{\%\ Ag > 0.25\,mm\,(th)}}{\sum \mathbf{\%Ag > 0.25\,mm\,(ts)}}$$

(Ag =aggregates; th= wet sieving; ts= dry sieving; mm= millimeters)

Statistical processing of results for each crop was performed using STATGRAPHICS 4.1 statistical package. Results on mycorrhizal colonization were evaluated (%) and transformed (arcos $\sqrt{\%}$) . All crop results followed a unifactorial variance analysis, whereas data of soil physics were evaluated on a factorial analysis.

RESULTS AND DISCUSSION

After analyzing mycorrhizal colonization in soybean-corn-sweet potato sequence (Table II), it is seen that, from the second crop, values reached on B2 inoculation frequence were significantly lower than the ones reached in frequencies presenting a higher intensity of inoculation (B3 and B4). This suggested that propagules in the soil were not enough for achieving similar values to the ones inoculated under these conditions. However, when the third

Table I. Main soil features at the beginning of the experimet

Depth (cm)	pH H_2O	OM (%)	P (ppm)	K^+	Ca^{++}	Mg^{++}	Density ($g.cm^{-3}$) Da	Dr	Kes	Keh	Ie
0-20	6.3	2.6	298	0.50	10.2	2.30	1.03	2.78	1.99	0.98	0.57
20-40	6.2	2.0	259	0.34	8.8	2.08	1.10	2.80	2.04	0.88	0.55

Da= Bulk density; Dr=Real density; Kes= Dry structure coeficient; Keh=humid structure coeficient Ie= stability coefficient crop (sweet potato) was planted, inoculating the first two crops was enough and, therefore, there was no need for inoculating the third sequence crop, since no significant differences were found between B3 and B4 frequencies. In this sense, it is pointed out that root colonization is affected by the inoculum dose used (8), as well as by its composition and distribution in the soil; whereas some researchers (9) found that both hyphae and spores present a high spatial and temporary variability.

Table II. Behavior of crop colonization and yield

Sequence		Colonization $arcos\sqrt{\%}$	Yield. ($t.ha^{-1}$)
1			
Soybean	B1	0.96 b	1.82
	B2	1.33 a	1.91
	B3	1.34 a	1.97
	B4	1.33 a	1.96
	SE x	0.05**	0.05 ns
Corn	B1	0.97 c	5.04 b
	B2	1.23 b	5.07 b
	B3	1.45 a	5.49 a
	B4	1.44 a	5.46 a
	SE x	0.05**	0.10**
Sweet potato	B1	1.14 c	18.50 b
	B2	1.44 b	18.80 b
	B3	1.68 a	21.20 a
	B4	1.68 a	22.70 a
	SE x	0.04**	0.62**
2			
Soybean	B1	0.91 b	1.92
	B2	1.42 a	1.95
	B3	1.35 a	1.93
	B4	1.36 a	1.94
	SE x	0.06**	0.03 ns
Sunflower	B1	1.04 c	1.60 b
	B2	1.58 b	1.69 a
	B3	1.79 a	1.71 a
	B4	1.78 a	1.75 a
	SE x	0.04**	0.03**
Sorghum	B1	1.01 c	1.90 ab
	B2	1.25 b	1.80 b
	B3	1.53 a	2.19 a
	B4	1.66 a	2.23 a
	SE x	0.08**	0.09**

Sequence 2 presented similar behavior and these results corroborate what has been previously found (10), where colonization increased in corn and sunflower when both crops were seeded after soybean. However, preceded by sunflower with high colonization, sorghum crop did not reach higher values. This indicates that value ranges achieved in such variable are directly related to crop species (11); they have stated that crop species differ markedly in their ability for responding to mycorrhizal symbiosis.

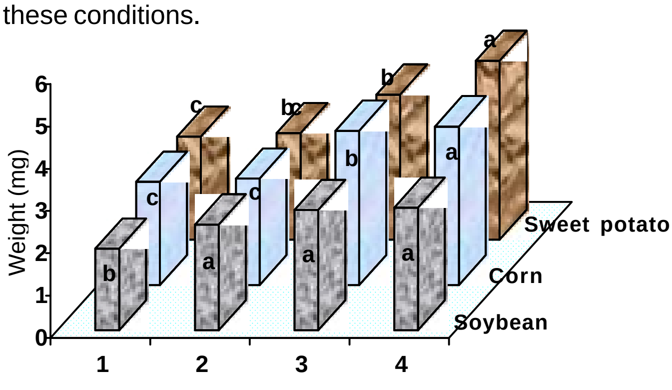
On the other hand, it has been pointed out (12) that, in relation to mycorrhizal dependence, sorghum is among low dependent crops, whereas corn, soybean and sunflower are included within the highly dependent group.

In both sequences, colonization achieved by check treatments increased when different crops were included, but the most dependent crops reached the highest values, even though such values were still very low for achieving a marked recolonization and influencing crop nutrition and development.

All this indicates the post-effect of mycorrhizal application on any crop, but effect intensity will depend on species features. On the other hand, tillage intensity in these systems could bring about propagule dilution in the soil or inactivation of some of them, due to soil prism inversion.

Yields achieved in soybean crop did not show significant differences in relation to the fertilized check, evidencing efficiency of coinoculation for nitrogen fixation and use. These results confirm that association of roots and AMF with bacteria is not random; on the contrary, they are associated by hierarchical structure of mutual preference (13). Moreover, some authors (14) proved that fungal hyphae contribute to mineral nitrogen acquisition and nitrogenase activity is superior in mycorrhized plants. At the remaining crops, the highest yields were achieved when, at least, the first two crops were inoculated in each sequence.

Figures 1 and 2 show values of endophyte weight, reached by crops in both sequences. According to results, the highest values were achieved by crops with superior colonization percentages, suggesting a good mycorrhizal functioning of all the crops used but sorghum, of which response to mycorrhizal inoculation was not high under these conditions.

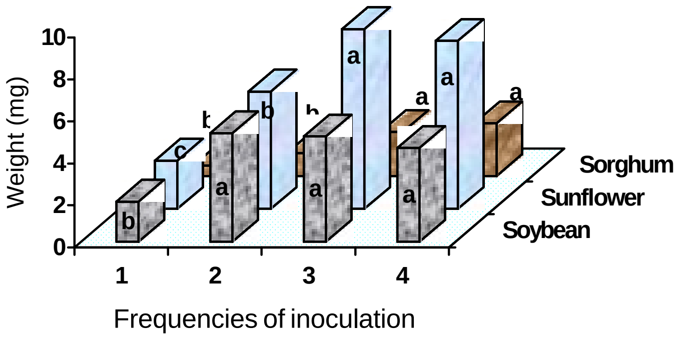


(SEx soybean= ± 0.38; SEx corn= ± 0.38; SE x sweet potato= ± 0.33)
1. check;
2- inoculation with AM only in soybean;
3- inoculation in soybean and corn;
4- inoculation in soybean, corn and sweet potato

Figure 1. Endophyte weight at the different frequencies of inoculation in sequence 1

There was also influence of plant-AMF-bacterium tripartite symbiosis on some indicators of soil physical properties. In this sense, even though significant differences were not found in real and bulk densities, there was neither significant interaction between cropping sequence and inoculation frequency, when the effects of treatments on other indicators were analyzed (Table III).

This allowed to perform independent factor analysis, obtaining significant differences in sequence factor, only for aggregate sizes between 2 and 5 mm. The highest values corresponded to soybean-sunflower-sorghum sequence, where soil was under root effect, favoring aggregate formation within such range of size, whereas frequency of inoculation showed significant differences in all parameters studied.



(SEx soybean= ± 0.51; SEx sunflower= ± 0.5; SEx sorghum = ± 0.44)
1- check;
2- inoculation with AM only in soybean;
3- inoculation in soybean and sunflower;
4- inoculation in soybean, sunflower and sorghum

Figure 2. Endophyte weight at the different frequencies of inoculation in sequence 2

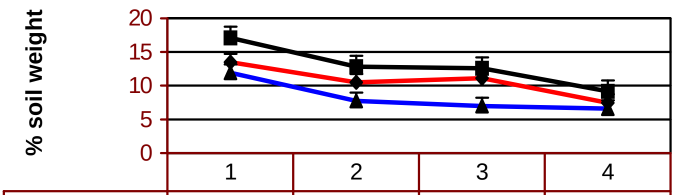
Table III. Behavior of some soil physical indexes

Factors		Parameters			Aggregate size		
		Micro-structure	KD	Ie	<1 mm	<2 mm	2-5 mm
Sequence	1	9.1	12.09	0.57	8.6	35.3	20.3 b
	2	9.3	12.07	0.58	7.0	33.4	29.2 a
	SE x	0.7 ns	0.42 ns	0.13 ns	0.7 ns	1.4 ns	1.8**
Frequency of inoculation	B1	12.9 a	17.90 a	0.54 b	11.3 a	39.7 a	17.8 c
	B2	8.8 b	12.06 b	0.55 b	8.4 ab	35.0 ab	23.4 bc
	B3	7.8 b	10.70 b	0.55 b	5.8 b	33.0 b	28.7 a
	B4	7.2 b	9.10 b	0.65 a	5.4 b	29.7 b	29.2 a
	SE x	1.0**	1.33**	0.02**	1.0**	2.0**	2.1**

The amount of clay in macroaggregates reduced in those treatments with a higher frequency of inoculation. A similar behavior was shown by smaller aggregates, characterizing the action of those microorganisms used in particle aggregation. It has been pointed out (6) that fungal hyphae are able to net soil particles and form bigger stable aggregates. Fungi are also able to produce glomalin, a glycoprotein that is placed in the external part of hyphae as well as on adjacent soil particles. This compound functions as a stable hydrophobic glue, which reduces aggregate rupture during dampness and drought, that are caused by a delayed movement of water within pores from aggregate structure. So, some authors (15) found a close relationship between aggregate stability and glomalin production by AMF, but such effect was superior in plots with minimum tillage. Similar relationship was found in pastures on soils exposed to high levels of weathering (16).

Inoculation reduced dispersion coefficient, at least in the first crop, whereas stability index increased significantly only in the variant where all sequence crops were inoculated. On the other hand, aggregate sizes between 2 and 5 mm were superior when two or three crops of the sequence were inoculated. These results coincide with others (17), that found a marked contribution of AMF to soil macroaggregate stabilization, the effect being significant after one growing season. Such effects were associated with the action of hyphae on the process of joining particles at first and in their further stability once aggregates were formed, as well as with the stimulation of plant root growth by AMF.

Figures 3 and 4 show the dynamics of clay percentage in microstructure, as well as of dispersion and microaggregate coefficients , which progressively reduced in both sequences, except for microaggregates in the second sequence, keeping a stable behavior. Reduction of dispersion coefficient showed that, with the biofertilization systems used in cropping sequence, the possibilities for soil to degrade are lower; therefore, its periods of productivity could be longer.

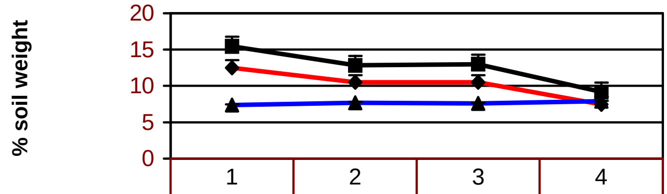


	1	2	3	4
Microest.	13.48	10.48	11.1	7.48
Coef.disp.	17.12	12.82	12.56	9.15
Agreg<.25	11.9	7.75	6.95	6.62

Biofertilization systems

1-check; 2-inoculation only in soybean; 3-inoculation in soybean and corn; 4-inoculation in soybean, corn and sweet potato

Figure 3. Dynamics of clay percentage in microstructure, dispersion as well as <0.25 mm aggregate coefficients in different biofertilization systems for sequence 1



	1	2	3	4
Microstruct	12.48	10.48	10.48	7.48
Avail. Coef.	15.45	12.82	12.98	9.15
Aggreg<.25	7.38	7.68	7.58	7.87

Biofertilization systems

(1-check; 2-inoculation only in soybean; 3-inoculation in soybean and sunflower; 4-inoculation in soybean, sunflower and sorghum)

Figure 4. Dynamics of clay percentage in microstructure, dispersion as well as <0.25 mm aggregate coefficients in different biofertilization systems for sequence 2

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