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Contribution of *Amynthas gracilis* (Megascolecidae) and *Octolasion cyaneum* (Lumbricidae) to soil physical stability: a mesocosm experiment

Contribución de *Amynthas gracilis* (Megascolecidae) y de *Octolasion cyaneum* (Lumbricidae) a la estabilidad física del suelo: una experiencia en mesocosmos

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ABSTRACT

The contribution of the introduced species *Amynthas gracilis* (Kinberg, 1867) and *Octolasion cyaneum* (Savigny, 1826) to the physical stability of the soil was evaluated in a mesocosm experiment. Pore formation and stable aggregates were measured; as well as changes in bulk density, porosity, and soil moisture. Mesocosm pots were organized into three treatments: 1- soil + *Amynthas gracilis*, 2- soil + *Octolasion cyaneum* and 3- soil (control containers). The experiment ran for 13 weeks and it was conducted in controlled conditions in a greenhouse. At the end of the experiment both treatments with earthworms had higher number of pores and stable aggregates at the two considered depths (0 - 5 cm and 5 - 10 cm). The presence of both earthworm species favors the formation of a significantly higher proportion of stable aggregates larger than 5 mm (60%), when compared to the control without worms. These structures helped maintaining bulk density and porosity and improved water circulation. The results show that when compared to the control, both treatments had a lower loss of pore space, lower bulk density, and higher soil moisture, all attributable to earthworm presence. It is concluded that, despite both being introduced species, in intensive agricultural systems, *A. gracilis* and *O. cyaneum* can contribute to the maintenance of soil physical stability thus helping to preserve the sustainability of agro-ecosystems, even if native species became rare or locally extinct.

Keywords
pores • soil physical stability • aggregate stability • introduced species • earthworms

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RESUMEN

En un experimento de mesocosmos se evaluó la contribución a la estabilidad física del suelo de las especies introducidas *Amynthas gracilis* (Kinberg, 1867) y *Octolasion cyaneum* (Savigni, 1826). Se midió la formación de poros y de agregados estables, así como cambios en la densidad aparente, porosidad y humedad del suelo. Los contenedores de los mesocosmos fueron organizados en tres tratamientos: 1- suelo + *A. gracilis*, 2. Soil + *O. cyaneum*, and 3. Soil (contenedores control). El experimento se realizó a lo largo de 13 semanas en condiciones controladas en invernáculo. Al final del experimento los tratamientos con ambas especies generaron poros y mostraron mayor proporción de agregados estables a las dos profundidades consideradas (0 - 5 cm y 5 - 10 cm). Ambas especies de lombrices facilitaron la generación de una proporción significativamente mayor de agregados mayores a 5 mm (60%), en comparación con los controles sin lombrices. Estas estructuras ayudaron a mantener la densidad aparente y la porosidad y mejoraron la circulación de agua. Los resultados muestran que, comparados con el control, ambos tratamientos tuvieron una menor pérdida de espacio de poros, menor densidad aparente y mayor humedad de suelo, todos atribuibles a la presencia de las lombrices. Se concluyó que, a pesar de ser ambas especies introducidas, en sistemas agrícolas intensivos *A. gracilis* y *O. cyaneum* pueden contribuir al mantenimiento de la estabilidad del suelo, ayudando así a preservar la sustentabilidad de los agroecosistemas, aun cuando las especies nativas puedan convertirse en raras o localmente extintas.

Palabras clave
poros • estabilidad física del suelo • estabilidad de agregados • especies introducidas • lombrices

INTRODUCTION

The main influence earthworms have on soil is the modification of soil structure through the creation of burrows and the production of casts. It is thus generally claimed that earthworms can contribute to the regeneration of compacted zones and this has been demonstrated under laboratory conditions (10). Earthworms create soil biogenic aggregates with very particular physical, chemical, and microbiological properties (29).

However, the overall effects of earthworms on soil and ecosystem functioning are not uniform, concerning aggregate formation in particular, which may vary according to their ecological categories, the particular species involved (4, 14, 26, 29), and land use history (25). Aggregate's formation is the result of soil particle reordering, flocculation, and cementing, and it is known to be mediated by soil organic carbon, biota activity, ionic bridge, and clay and carbonates (7).

The increase in land-use intensity results in a decline of soil biodiversity (11, 21, 25, 30, 32, 41). Earthworms have been shown to be sensitive to the changes introduced by agricultural management (crop type, mineral nitrogen input, organic nitrogen input, mechanical field operations, and pesticide applications), which lead to the disappearance of native species or to their replacement by introduced species.

These changes have an impact on the ability of the soil to provide ecosystem services (3, 7, 12, 38). It is therefore important to know the contribution made by different earthworm species (4, 5, 26, 32). In this context it also becomes relevant to know the effects introduced species have in replacing native ones under changing conditions. Because most introduced species can tolerate a wide range of soil and environmental conditions, they have been often considered as the predominant earthworm fauna in anthropic tropical ecosystems (22), and to be important in maintaining the fertility of agricultural lands (31).

Amynthas gracilis (Kinberg, 1867) (Megascolecidae) and *Octolasion cyaneum* (Savigny, 1826) (Lumbricidae) are two peregrine introduced species sharing environments in Argiudoll soils of the rolling Pampas in Argentina. They have been successful in colonizing disturbed environments (18, 20, 35, 36, 37). Due to their wide distribution, it is important to know whether these two particular species, have a significant impact on soil physical properties. According to the classification by Bouché (1977), *A. gracilis* is an epi-endogeic species associated with litter (19), while *O. cyaneum* is an endogeic species living within the soil feeding on a mixture of organic matter and mineral soil.

This study focuses on evaluating changes in soil physical variables, brought about by the presence of these two earthworm species, comparing each one of them with the control. A temporal analysis of each treatment was also performed.

The working hypothesis was that *A. gracilis* and *O. cyaneum* presence contributes to soil physical stability. In this context, the objective of this work was to evaluate *A. gracilis* and *O. cyaneum* activity, through their effects in the generation of pores, number of stable aggregates, and its effects on soil bulk density, porosity, and soil moisture. The study also expected to find differences in the action of *A. gracilis* and *O. cyaneum*.

MATERIALS AND METHODS

Adult earthworms and soil were collected from cattle-grazing fields, on typical Argiudoll soils (18.6% clay, 63% silt, 18.4% sand, 3.6% organic matter, and 2.12% organic carbon).

The soil was sieved through a 2 mm mesh, to remove structure, homogenize the soil, and to extract all roots, and then dried at 30°C to constant mass. Sixty three mesocosm pots were each filled with 600 g of soil and were slowly irrigated. After 24 hours, they were further irrigated to saturation, left to drain to field capacity and weighed again. Thus, at the start of the experiment each container presented the same amount of soil and moisture condition. Each container within a treatment was then considered a replicate. The mesocosm pots were arranged in a greenhouse into three treatments: 1- soil + *Amynthas gracilis*, 2- soil + *Octolasion cyaneum* and 3- soil without earthworms (control treatment).

One adult of either one of the species was placed in each one of the 21 containers per treatment, and 50 g of manure was added as food. The control containers were processed in the same way but without earthworms. Throughout the experiment, temperature was monitored (23°C ± 2°C) and the containers were irrigated with 120 ml distilled water three times a week to keep them close to field capacity.

On each sampling date (30 and 90 days) seven random mesocosm pots per treatment were removed and dried in an oven at 30°C. In each container, the number and size (diameter) of the pores generated by each of the species was determined.

In order to do this, once the containers had been removed from the oven and their weight registered, a longitudinal cut was performed on the soil clod, and the diameter of each pore was measured with a caliper over one of its faces.

The wet method recommended by Ester and van Rozen (2012) was used to evaluate the stability of soil aggregates at two depths: from surface to 5 cm and from 5 to 10 cm. The wet procedure consisted in a continuous water flow from a shower tap on to a metal sieve column (5, 4, and 2 mm mesh diameters) filled with a 200 g soil sample. The rate of water flow was equalized for each replicate, and the samples remained for 2 min under the tap. The remaining material on the sieve was considered to be stable aggregates, expressed as the percentage by weight of the original sample. The aggregate sizes tested were > 5 mm; > 4mm, and > 2 mm.

At 0, 30, and 90 days, bulk density was determined according to Porta's methodology (42); total porosity was calculated from bulk density data.

Particle density was considered to be equal to the theoretical value for quartz (2.65 g/cm^{-3}), quartz being one of the most common soil mineral components. Soil moisture was evaluated through gravimetric humidity, calculated as the ratio of the mass of the liquid fraction to the mass of the solid fraction Gh (g of water * g of soil $^{-1}$) = wet weight - dry weight) / dry weight) following Gil (2002).

To test for significant differences and due to the non-normal distribution of the data, Kruskal -Wallis ANOVA was used to analyze the data. To identify which group or groups were significantly different, the Mann-Whitney test was used.

Results and Discussion

Pore number and size

Given the conditions under which the test was developed (dried and sieved soil), all pores observed in the pots are part of the galleries created by earthworms. The statistical analysis shows that the number and size of pores varied between treatments ($p < 0.05$), (table 1, page 119) and the number of pores clearly reflected earthworm activity.

Furthermore, *A. gracilis* had higher activity than *O.cyaneum*, as shown by the higher number of pores produced by this species ($p < 0.05$), (table 1, page 119). *A. gracilis* differed from *O. cyaneum* not only in the amount of pores (greater in both periods), but also in their size distribution. Significant differences were found for 3, 4 and 5 mm pores. While the number of pores indicates movement in the container, the diameter of the pores reflects earthworm body size. Most of the pores built by *A. gracilis* ranged from 3 to 4 mm in diameter, ($p<0.05$) while most of the pores built by *O. cyaneum* were 2 mm in diameter (table 1, page 119). These differences were maintained throughout the experience.

The existence of these biopores, largely regulates soil hydraulic properties and affect soil organic matter turnover (29). Macroporosity (pore diameter greater than 1 mm), promotes water infiltration into the soil allowing for a fast flow of gravitational water (35). All the pores found in this study were larger than 1 mm in diameter, which facilitates the process of incorporating water into the soil, and enhancing soil moisture.

Table 1. Number of pores by size and aggregate formation in the treatments with *Amynthas gracilis* and *Octolasion cyaneum* in a mesocosm experiment.

Tabla 1. Número de poros según tamaño y formación de agregados en los tratamientos con *Amynthas gracilis* y *Octolasion cyaneum* en un experimento de mesocosmos.

	Pores					**Total aggregates (%)**	**aggregates (%) by size at the two depths**					
		size					**>2mm**	**>4 mm**	**>5mm**	**>2 mm**	**>4 mm**	**>5 mm**
	Number of pores *mesocosm^{-1}	**2 mm**	**3 mm**	**4 mm**	**5 mm**		0 - 5 cm			5 - 10 cm		
day 30												
Amynthas gracilis	10 +/-3 Aa	3 +/-4 ADa	3 +/- 2 BDa	4 +/-2 ADa	1 +/- 1ADa	50 Aa	14.9 ALGa	7.3 AKGa	27.3 BJGa	8.5 AKGa	6.2 AKGa	35.8 AJGa
Octolasion cyaneum	3 +/- 3 Ab	3 +/-3 ADa	0 BEb	0 AEb	0 AEa	60 Aa	19.6 AJGa	11.3 AJGa	18.5 BJGa	12.2 AKGa	7.5 AKGa	30.9 AJGa
control	0 Bc	0 BDb	0 ADb	0 ADb	0 ADa	30 Ab	27.6 AJGa	16.2 AKGa	12 BKGb	21.3 AJGa	10.7 AKGa	12.2 AJGb
day 90												
Amynthas gracilis	14 +/-3 Ba	1 +/-1 AEa	6 +/-3 ADa	5 +/-3 ADa	2 +/- 2AFa	60 Aa	10.3 AKGa	8.1 AKGa	30.1 AJGa	10.8 AKGa	6.8 AKGa	33.4 AJGa
Octolasion cyaneum	4 +/-2 Ab	3 +/-2 Ab	1 +/-1 AEb	0.5 +/- 1AEb	0 AEa	70 Aa	11.9 BLGa	6.6 BKGa	35.6 AJGa	6.7 BKGa	6.3 AKGa	32.9 AJGa
control	1 +/-1 Ac	1 +/-1 ADa	0 ADb	0 ADb	0 ADa	30 Ab	22.8 AJGa	8.7 BKGa	20.6 AJGb	21 AJGa	11.1 AKGa	16.5 AJGb

Kruskal Wallis ANOVA test and post hoc Mann–Whitney U-test. Means ± standard deviation are shown for the pore size testing. Different lower case letters abc in the same column and date indicate significant differences between treatments ($p<0.05$). Uppercase letters indicate significant differences ($p<0.05$) according to the following combinations: ABC letters used for differences between dates within the same treatment (upper and lower parts of the table); only for pore size (left hand side of the table) DEF letters used for pore number differences according to size within treatments; only for % aggregates (right hand side of the table) JKL letters used for differences according to size within treatments at the same depth; GHI letters used for differences in % aggregates of the same size between the two depths.

Test de Kruskall-Wallis y Mann-Whitney post-hoc. Se muestran las medias ± desviación estándar para tamaño de poros. Diferentes letras minúsculas abc en la misma columna indican diferencias significativas entre tratamientos ($p<0,05$). Letras mayúsculas indican diferencias significativas ($p<0,05$) de acuerdo con las siguientes combinaciones: ABC usadas para diferencias entre fechas, dentro del mismo tratamiento (partes superior e inferior de la tabla); solo para tamaño de poros (lado izquierdo de la tabla) DEF usadas para diferencias en el número de poros según tamaño; solo para % de agregados (lado derecho de la tabla) JKL usadas para diferencias en % de agregados según tamaño para un mismo tratamiento y profundidad; GHI usadas para diferencias en % de agregados del mismo tamaño entre ambas profundidades.

Aggregate formation

Because of its role in the retention of organic carbon and moisture, soil aggregation is an important factor for sustainable land management (1), and earthworms are facilitators in the process of soil aggregation production and maintenance. Earthworm mixing of organic matter with mineral soil has significant effects on the production of aggregates and on their properties (1, 8).

The data gathered show significant differences on stable aggregates' found ($p<0.05$) between the treatments with earthworms and the control (table 1, page 119) during the whole experiment. This result supports the notion that organic input and earthworm activity play a significant role in promoting soil aggregation (2). This study is in agreement with the results obtained by Mummey *et al.* (2006), working with *Aporrectodea caliginosa*, and those by Bossuyt *et al.* (2006) with *A. caliginosa* and *L. rubellus*.

After 30 days, the treatments with both *A. gracilis* and *O. cyaneum* showed a large proportion of stable aggregates (50% and 60% respectively).

However, by day 90 the increase in aggregate found was lower (for a total of 60% for the treatment with *A. gracilis* and 70% for the treatment with *O. cyneum*), with no significant increase in the amount of aggregate formation in either treatment.

In this regard, Al-Maliki and Scullion (2013), reported that their treatment with *Lumbricus terrestris* showed significantly more aggregates up to day 40, and that earthworm induced increases in stable aggregates were not significant from day 40 to day 80.

However, these authors also found that the differences between treatments and the control were lost, while the ones presented in this study remained the same until the end of the experiment.

There were also significant differences in the number of stable aggregates between the treatments with the two species. The treatment with *A. gracilis*, showed a higher proportion of stable aggregates larger than 5 mm at both depths analyzed (0- 5 cm and 5 -10 cm) and the relationships between aggregate sizes (5 mm> 2 mm> 4 mm) also remained the same ($p < 0.05$). The amount of larger aggregates in the first 5 cm increased from day 30 to day 90 at the expense of the smaller ones ($p < 0.05$).

The treatment with *O. cyaneum* also showed a higher proportion of aggregates larger than 5 mm, and it was also higher than that of >2 to >4 mm ($p<0.05$). At day 30, > 5mm aggregates were at the 10 cm ($p <0.05$). By the end of the experiment, the proportion of these stable aggregates was the same at both depths. However, it is interesting to note that, while the proportion of larger aggregates increased with time ($p<0.05$), that of smaller ones decreased.

The low proportion of small aggregates at the end of the experiment, suggests that some of the smaller aggregates were incorporated into the larger ones. This was evident for the treatment with *O. cyaneum*, that by the end of the experiment showed an increase in aggregates larger than 5 mm and a decrease in smaller ones. Working with *Metaphire posthuma*, Jouquet *et al.* (2013) found a clear relationship between the sizes of the aggregates and the abundance of bacteria and fungi within them.

Mummey *et al.* (2006), found that microbial communities within aggregates, especially larger ones, varied depending on the presence of earthworms and that, within micro-aggregates formed by earthworms, micro-organisms were largely inactive due to the rapid occlusion of organic materials.

The results presented here provide evidence that the presence of *A. gracilis* and especially *O. cyaneum* promotes the formation of such larger aggregates.

Mummey *et al.* (2006), also showed that the abundance of bacteria and fungi in casts increased with the size of the aggregate and that the opposite trend was observed for the control. In laboratory experiments, Bossuyt *et al.* (2006), found that stable microaggregates were formed within 12 days of incubation in the presence of earthworms.

Hedde *et al*. (2013), found that, the structural stability of large aggregates was strongly influenced by the type of soil and earthworm species.

In the control treatment, aggregates were also formed, in this case the most frequent size was less than 2 mm ($p<0.05$). Other studies found similar results, calling them physicogenic aggregates (23, 27).

Malik and Scullion (2013), associate the formation of these aggregates to the activity of microorganisms (especially fungi); Jouquet *et al.* (2013), found that the intervention of bacteria and fungi was important in the formation of these physicogenic aggregates.

Jouquet (28) proposed that their formation is the result of both abiotic (till age processes, wetting and drying cycles) and biotic (microbial activity associated with root exudation and degradation but not soil macrofauna activity) processes.

Inside the aggregates, natural drying and time make the link between organic matter, minerals and mucus more resistant, leading to an increase in their stability (9, 44).

Shipitalo and Protz (1989) describe how earthworms directly promote the formation of organic matter-cored microaggregates.

Shaw and Pawluk (1986) and Shipitalo and Protz (1988) state that the proportion of aggregates of different sizes and their stability strongly depend on earthworm species and soil type.

Both *A. gracilis* and *O. cyaneum* promote stable aggregate formation of equal size and in similar proportions in the entire volume of the containers. No differences were found for the two depths (0 -5 and 5 to 10 cm).

In agreement with the data obtained in this study, Scullion and Malik (2000) found (for *Lumbricus terrestris, L. Rubellus, Aporrectodea longa, A. caliginosa* and *A. chlorotica*), both in microcosms and field studies, that aggregates are generally found between 0 and 20 cm deep, they range in size from 2 to 6.3 mm and that furthermore, these formations are more stable than other aggregates of non- biological origin.

Regarding the stability of biological aggregates, Marinissen and Dexter (1990); Marinissen (1994) and Shipitalo and Protz (1989) indicate that aggregates are unstable while fresh, but that they are more stable than non-biological soil aggregates once dry. This is reflected in the number of stable aggregates found in this study.

Both earthworm treatments had a significantly higher proportion of aggregates when compared to the control ($p<0.05$), and no differences were found between the size of the aggregates produced by each one of them.

Jouquet *et al.* (2008) state that aggregation is a major component of soil fertility, controlling porosity, water storage and oxygen diffusion, and therefore the mineralization or sequestration of soil organic matter (SOM) and the retention or the leaching of mineral nutrients. These studies showed that the presence of some of these species acted in favor of the system's stability.

Bulk density

The results show that the earthworm activity can maintain or change bulk density as well. In this study, density values measured at days 0, 30, and 90 of the experiment were compared (table 2, page 123).

Both earthworm species differ from the control ($p<0.05$) in which density had increased by 40% at the end of the experiment. These differences can be attributed to earthworm movement inside the container, organic matter incorporation, mixing it with the soil, casts production, and the formation of gallery systems and thus, reducing soil compaction.

No differences in bulk density were observed for *A. gracilis* or for *O. cyaneum* between dates. Regarding the control, bulk density increased ($p <0.05$) by the end of the experiment with no differences between the values between day 30 and day 90.

It is interesting to note that by day 30, the highest proportion of aggregates larger than 5 mm found in the *O. cyaneum* treatment were found at 5-10 cm, while for the *A. gracilis* treatment their distribution was homogenous for both depths. This difference in the location of the aggregates in each treatment could determine the differences in this variable, since by the end of the study both earthworm treatments had equal proportion and distribution of aggregates and the same response for the bulk density value.

These results also show that organic matter presence on the surface did not, by itself, ensure that the initial conditions were maintained. Irrigation determined a particle reorganization which generated a loss of porosity. Riley *et al.* (2008) mentions the same increase in density and the reduction of soil moisture in the absence of earthworms.

Doran and Parkin (1994) suggest that bulk density may be included among the minimum indicators used to measure soil quality. The results of this study indicate that, by maintaining bulk density, these two introduced earthworm species contribute to the preservation of soil stability.

Porosity and soil moisture

Preventing the loss of soil pore space ensures the maintenance of air and water flows as well as the soil's water retention capacity and rainwater reservoir (39).

The data gathered in this work indicate that, at the end of the study, the presence of *A. gracilis* or *O. cyaneum* prevented compaction, and differed from the control ($p<0.05$) (table 2, page 123).

Data shows that at day 30, only *A. gracilis* kept the porosity in the mesocosm. By that date, the *O. cyaneum* treatment, as well the as control, showed a porosity reduction and differed significantly to that of *A. gracilis* ($p <0.05$).

However, after 90 days, *O. cyaneum* increased the value of porosity, matching *A. gracilis* (62%), while the control reduced the value of this parameter even more (51%**)**. The control treatment showed a significant 7% reduction in porosity by day 30 ($p <0.05$) and an additional 8% reduction by day 90.

Jouquet *et al.* (2012) and citations thereof, agree that by creating burrows and egesting casts, earthworms modify soil porosity. Hedde *et al.* (2013) found that their activity increases incorporation of plant residues into soil aggregates, and enhance soil porosity and stable aggregates formation through the soil profile.

Table 2. Earthworm presence effects on soil physical parameters in a mesocosm experiment.

Tabla 2. Efectos de la presencia de las lombrices sobre parámetros físicos del suelo en un experimento de mesocosmos.

	bulk density (g * cm^{-3})			**Porosity (%)**			**Gravimetric humidity (g of water * g of soil $^{-1}$)**		
	day 0	day 30	day 90	day 0	day 30	day 90	day 0	day 30	day 90
Amynthas gracilis	0.9 +/- 0.04 **A**	0.9 +/- 0.1 **Aa**	1 +/- 0.1 **Aa**	66 +/- 1.5 **A**	63 +/- 2.7 **Aa**	62 + /- 2 **Aa**	0.22 +/- 0.04 **A**	0.42 +/- 0.1 **Ba**	0.34 +/- 0.03 **Ca**
Octolasion cyaneum	0.9 +/- 0.03 **A**	1.1 +/- 0.2 **Aab**	1 +/- 0.03 **Aa**	65 +/- 1.2 **A**	58 +/ - 8 **Ab**	62 + /- 1.5 **Aa**	0.23 +/- 0.03 **A**	0.39 +/- 0.2 **Ba**	0.37 +/- 0.03 **Ba**
Control	0.9 +/- 0.03 **A**	1.2 +/- 0.2 **Bb**	1.3 +/- 0.2 **Bb**	66 +/- 1.1 **A**	59 +/- 7 **Bb**	51 +/- 8 **Bb**	0.23 +/- 0.03 **A**	0.2 +/- 0.1 **Ab**	0.2 +/- 0.1 **Ab**

Kruskal Wallis ANOVA test and post hoc Mann–Whitney U-test. Means ± standard deviation are shown. Different lowercase letters in the same column indicate significant differences between treatments ($p<0.05$). Different capital letters in the same row indicate significant differences in the same treatment ($p<0.05$)

Test de Kruskal-Wallis y Mann-Whitney test. Se muestran medias ± desviación estándar. Diferentes letras minúsculas en la misma columna indican diferencias significativas entre tratamientos ($p<0,05$). Diferentes letras mayúsculas en la misma columna indican diferencias significativas dentro del mismo tratamiento ($p<0,05$).

The results presented here indicate that porosity is influenced by the presence of aggregates and pores. The control showed a lower proportion of aggregates when compared with both treatments (table 1, page 119), albeit not enough to avoid compaction and loss of pore space. In the case of the *A. gracilis* treatment, the combination of aggregates found both in number and in size, and the higher number of pores by day 30, allowed for a significant effect in the maintenance of soil porosity, a condition that remained high until the end of the trial.

In the case of the *O. cyaneum* treatment, the low amount of pores and a lower proportion of aggregates larger than 5 mm may be the reason for the loss of porosity observed by day 30. However after 90 days, the higher proportion of large aggregates lead to the observed increase in porosity at the end of the experiment.

Even though earthworm burrows make up a small portion of the total soil porosity (14, 30), their relatively large size and connectivity make them important conduits for water and air movement (30), and can alter soil hydraulic conductivity (15, 28).

Regarding soil humidity, both mesocosms with earthworms showed higher water content when compared to the control ($p < 0.05$) throughout the entire study. No differences were found when comparing the treatments with *A. gracilis* and *O. cyaneum* (table 2, page 123).

Soil humidity in each treatment also varied over time for both earthworm treatments, with no change for the control. The mesocosm with *A. gracilis*, showed significant differences between dates. Up to day 30, the water content significantly increased from 0.22 g of water per gram of soil to 0.42 g of water per gram of soil ($p<0.05$) and then decreased to 0.34 grams of water per gram of soil by day 90, but still remained higher than at day 0 ($p<0.05$). *O. cyaneum*, also increased the water content after 30 days, from 0.23 to 0.39 grams of water per gram of soil ($p<0.05$), with no further significant change by day. In the treatment without earthworms soil humidity showed no change throughout the 90 days of the study.

These results differ from those found by Eltaif and Gharaibeh (2009) who found a reduced hydraulic capacity in experiments with *Aporrectodea caliginosa* in mesocosm conditions. This supports the idea that an earthworm effect on soil properties is species-dependant, and related to behavior and life strategy.

CONCLUSIONS

This study showed significant changes in soil physical variables brought about by the presence of these two earthworm species. The results presented support the working hypothesis that *A. gracilis* and *O. cyaneum* presence contributes to soil physical stability. The presence of both species had significant effects in the generation of pores, number of stable aggregates, soil bulk density, porosity, and soil moisture. There were also significant differences in the action of *A. gracilis* and *O. cyaneum*.

The results of this study show the important effects that both *A. gracilis* and *O. cyaneum* have on the formation of aggregates and pores, with significant effects on soil structure through the variables measured.

Through its effects in the mesocosm in the generation of pores and the promotion in the formation of aggregates of different sizes that were located at both depths, *Amynthas gracilis* showed a positive effect on the measured variables. The resulting structure allowed for the maintenance of a higher porosity and a resulting lower bulk density. These effects of *A. gracilis* behavior remained until the end of the study. For *O. cyaneum* the positive effect was observed at the end of the study (day 90), preventing compaction and loss of pore space.

The differences found between both earthworm treatments suggests that the location of the aggregates has significant effects on bulk density and porosity.

In agreement with the cited literature, it can be concluded that small amount of aggregate formation in the control was the result of particle reorganization, cementation, and participation of microorganisms. However, the significantly higher aggregate formation and higher porosity in the treatments with either earthworm species, as well as the lower resulting bulk density and higher soil humidity, all highlight the importance of these earthworm species.

Therefore, the presence of these introduced species is shown to contribute to the maintenance of soil stability.

The results presented in this work mean that, in environments in which one or both of earthworm species are present, compaction processes will not show, even if native species became rare or locally extinct. As these two species are introduced to the region, this is a particularly important result, showing that introductions need not be detrimental to the system.

Understanding the contributions made by each species individually, gives us a tool to use not only as an indicator of environmental conditions but also for the development and implementation of soil recovery systems. Carrying out field experiments may help to corroborate the results obtained in this study under laboratory conditions.

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