Reprinted from

Applied Soil Ecology

A section of Agriculture, Ecosystems & Environment

Applied Soil Ecology 3 (1996) 177–181

Short communication

Vesicular–arbuscular mycorrhizal fungi in earthworm casts and surrounding soil in relation to soil management of a semi-arid tropical Alfisol *

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Accepted 29 September 1995

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A section of Agriculture, Ecosystems & Environment

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Publication Information. *Applied Soil Ecology* (ISSN 0929-1393). For 1996 volumes 3–4 are scheduled for publication. Subscribers to *Agriculture, Ecosystems and Environment* will have the opportunity to subscribe to *Applied Soil Ecology* at a special reduced subscription rate. Subscription prices are available upon request from the Publisher. Subscriptions are accepted on a prepaid basis only and are entered on a calandar year basis. Issues are sent by surface mail except to the following countries where air delivery via SAL mail is ensured: Argentina, Australia, Brazil, Canada, Hong Kong, India, Israel, Japan, Malaysia, Mexico, New Zealand, Pakistan, PR China, Singapore, South Africa, South Korea, Taiwan, Thailand, USA. For all other countries airmail rates are available upon request. Claims for missing issues should be made within six months of our publication (mailing) date. Please address all your requests regarding orders and subscription queries to: **Elsevier Science B.V., Journal Department, P.O. Box 211, 1000 AE Amsterdam, The Netherlands, Tel. (+31-20)4853642, Fax (+31-20)4853598.**

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Abstract

Spores and infective propagules of vesicular-arbuscular (VA) mycorrhiza were examined in earthworm casts and field soils collected from three different soil management treatments (zero tillage with straw amendment, deep tillage without amendment (bare), and zero tillage with previous perennial cropping of *Stylosanthes hamata*) on an Alfisol in the semi-arid tropics (SAT) of India. The average mean of spore counts and most probable number (MPN) of infective propagules of VA mycorrhiza (VAM) were significantly ($P < 0.05$) higher in earthworm casts than in field soil across the three soil management treatments. There was no significant difference in the number of VAM spores or propagules among field soils from the three different soil management treatments, but the number of VAM spores and propagules in the earthworm casts from the deep tillage bare treatment was significantly higher ($P \leq 0.05$) than in the earthworm casts from the other two treatments. In the deep tillage bare treatment, the number of spores and MPN of infective propagules were significantly ($P < 0.05$) higher in earthworm casts than in field soil. Therefore, it may be concluded that earthworms can concentrate VA mycorrhizal spores and propagules in their casts.

Keywords: Earthworm casts; Soil management; VAM propagules; Vesicular-arbuscular mycorrhiza

1. Introduction

Vesicular-arbuscular (VA) mycorrhizal fungi occur in nearly all soils. The symbiosis between host plant roots and VA mycorrhiza (VAM) is widely known to be important to the nutrient acquisition of host plants (Barea, 1991). Owing to the hypogeous nature of VA mycorrhiza and because it is symbiotic, VA mycorrhiza requires mobile vectors or living roots for its translocation and dispersal. A wide range of vertebrates and invertebrates have been suggested as potential vectors of VA mycorrhiza (McIlveen and Cole, 1976; Harinikumar and Bagyaraj, 1994), and earthworms are considered to be important in its dispersal (Rabatin and Stinner, 1986).

Earthworms can transport a large amount of soil

* Submitted as ICRISAT Journal article No. 1820.

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SSDI 0929-1393(95)00082-8

through their feeding activity (Lavelle et al., 1983), and therefore can disseminate both beneficial and detrimental soil microorganisms in the upper part of the soil profile or to the surface of the soil. The soil passed through the gut of earthworms is different from the field soil in its qualitative and quantitative composition of microorganisms (Doube et al., 1994). It has recently been demonstrated that earthworms concentrate VA mycorrhizal propagules in their casts (Reddell and Spain, 1991; Gange, 1993). This is important for an agricultural ecosystem because earthworms and VA mycorrhiza can jointly contribute to enhanced soil fertility and crop nutrition.

Agricultural practices such as soil management can greatly influence the population size and activity of both VA mycorrhiza and earthworms. The present study aims to demonstrate that earthworms concentrate the spores and propagules of VA mycorrhizal fungi in their casts, and to quantify the spores and viable propagules in earthworm casts and in non-ingested soils as influenced by the soil management treatments on a semi-arid tropical Alfisol in India.

2. Materials and methods

The experimental site was an Alfisol at the ICRISAT Asia Center, Patancheru, Andhra Pradesh, India. The details of the site have been reported by Smith et al. (1992). The experiment had 15 soil management treatments with three replications. This included nine annually cropped treatments comparing tillages (zero, 10 cm and 20 cm) and mulches (no mulch, farm yard manure and rice straw), and six perennials: pigeonpea (*Cajanus cajan*) (P), *Cenchrus ciliaris* (C), *Stylosanthes hamata* (S), and mixtures of these species, i.e. PS, PCS and CS. In 1993, sorghum (*Sorghum bicolor* (L.) Moench) was planted in all the treatments to investigate previous treatment effects. Three soil management treatments (three

treatment showed the largest number of casts among annual crop treatments, while the previous cropping of *S. hamata* treatment showed the largest number of casts and the deep tillage bare treatment produced medium numbers of casts among all the treatments (Reddy et al., 1995).

As the earthworm biomass was highest in August and September during 1989–1992 (Reddy et al., 1995), earthworm casts and field soil samples were collected on 16 August 1993. Field soil samples were randomly collected from six spots 10 cm deep in each replication of each treatment, and mixed thoroughly by shaking in a plastic bag. Earthworm casts were also randomly collected from six spots in each replication of each treatment, and mixed in a plastic bag.

Soils and casts (200 g per replicate) were used to extract the VAM spores by a wet-sieving method (Gerdemann and Nicolson, 1963). The most probable number (MPN) of infective propagules was estimated as described by Porter (1979). A 10-fold dilution series was first made by mixing 10 g soil or casts for each replicate of treatment with 90 g diluent sterilized sand. The second dilution was made by mixing 10 g of the mixture of soil or cast and sand from the first dilution with 90 g diluent sterilized sand. This dilution was done to 10^{-5} with five replicates. Then 10 g of 10^{-3}, 10^{-4} and 10^{-5} dilution soils were placed in a plastic pot containing 350 g sterilized sand. Three sorghum seeds were sown in one pot, and the seedlings were thinned to one a week later. The plants were harvested after 6 weeks under glasshouse conditions, where they were maintained by periodically supplying nutrient solution devoid of phosphorus. The sorghum roots were washed free of soil, autoclaved in 10% KOH, and stained in trypan blue–locto–glycerol. Infection was observed under 40 × magnification, and the MPN was calculated as described by Alexander (1982).

The average spore and propagule numbers were
... treatment. The standard error of
means under different groups of
ıtained from GENSTAT analysis
The least significant differences
ıted using these standard error of
as given in Cochran and Cox

3. Results and discussion

The density of VA mycorrhiza spores and the MPN of infective VA mycorrhiza propagules in earthworm casts and in field soils are shown in Table 1. Two species of endogeic earthworm, *Ochochaetona phillotti* (Michaelsen) and *Lampito mauritii* (Kinberg), were recorded at the experimental site, the former being dominant. The abundance of earthworms per square metre was 6, 59 and 40 for deep tillage bare, zero tillage with added straw and previous cropping of *S. hamata*, respectively (Reddy et al., 1995). The casts of both types of earthworm were small rounded pellets and were morphologically indistinguishable. The grand mean of the spore number in the casts across the three soil management treatments was significantly higher ($P \leq 0.05$) than that in the field soils. This indicates that, overall, earthworm casts contain significantly higher densities of spores than field soils. The average mean number of spores in earthworm casts and field soil from deep tillage bare treatments was significantly ($P \leq 0.05$) higher than that from other soil management treatments. The earthworm casts from the deep tillage bare treatment had significantly higher ($P \leq 0.05$) numbers of spores than those from the other two treatments. However, no significant difference in the spore numbers was found among the three field soils from different soil management treatments. The extent to which earthworms increase the number of spores was greater in deep tillage bare treatments (2.4-fold) than in zero tillage with straw treatments (1.6-fold), but there was no increase in previous cropping of *S. hamata*.

The MPN values are much higher than those reported from locations in other parts of the world (Table 1) (e.g. An et al., 1990). However, the values are comparable to those reported from another Indian soil (Harinikumar and Bagyaraj, 1988). As with the density of spores, overall, the density of propagules

Table 1
VA mycorrhiza spores and MPN of infective VA mycorrhiza propagules from earthworm casts and field soil

Soil management	No. of spores (g^{-1} dry soil)			
	Casts		Soil	Mean
Deep tillage bare	63		26	45
Zero tillage with added straw	23		14	19
Previous *S. hamata* (zero tillage)	21		19	20
Mean	36		20	
LSD (1) [a]		9.8		
LSD (2) [b]		17.4		
LSD (3) [c]				25.6
LSD (4) [d]		27.5		
	MPN of propagules (g^{-1} dry soil $\times 10^2$)			
Deep tillage bare	102.0		23.3	62.7
Zero tillage with added straw	24.0		11.7	17.9
Previous *S. hamata* (zero tillage)	16.7		3.1	9.9
Mean	47.6		12.7	
LSD (1) [a]		20.79		
LSD (2) [b]		34.33		
LSD (3) [c]				40.50
LSD (4) [d]		48.34		

[a] To compare grand means of casts and soil at $P = 0.05$.
[b] To compare means of a pair within a soil management at $P = 0.05$.
[c] To compare means of soil managements.
[d] To compare mean values of any pair from the casts and/or the field soils at $P = 0.05$.

across three soil management treatments was higher in earthworm casts than in field soil. The average mean number of propagules in earthworm casts and field soil from the deep tillage bare treatments was significantly ($P \leq 0.05$) higher than that from other soil management treatments. The earthworm casts from the deep tillage bare treatments had significantly higher ($P \leq 0.05$) numbers of propagules than those from the other two treatments. However, no significant difference in the propagule numbers was found among the three field soils from different soil management treatments. Within a soil management treatment, deep tillage bare treatments showed a significant difference ($P \leq 0.05$) in the density of propagules between earthworm casts and field soil, with a 4.4-fold increase when earthworms are present. The earthworm casts from the deep tillage bare treatments contained the highest density of propagules, and the lowest densities of propagules were found in the field soil of previous cropping of *S. hamata*.

VA mycorrhiza infection of sorghum was examined in eight selected treatments in an earlier study using the same experimental plots (Lee et al., 1995). The infection was highest with sorghum roots in deep tillage bare treatments, and lowest with previous cropping of *S. hamata* treatments and previous cropping of *Cenchrus ciliaris* treatments, both of which received zero tillage treatment. It may be assumed from the present study and the earlier one that earthworms feeding on decaying roots high in mycorrhiza infection produce casts containing high densities of spores and propagules.

We assume that a lower infection rate and smaller numbers of propagules of VA mycorrhiza are due to zero tillage, and this is supported by the results that deep tillage bare treatments showed the highest infection rate and greatest numbers of propagules of VA mycorrhiza. In contrast to our observations, other studies have demonstrated that soil disturbance such as tillage reduced VA mycorrhizal infection compared with zero tillage (Fairchild and Miller, 1988). At present, we cannot explain why deep tillage increased the infection and the number of VA mycorrhiza. However, it may be possible that tillage practice (soil disturbance) helped in the dispersal of infective hyphae or propagules.

These results confirm that earthworm casts contain considerably higher numbers of VA mycorrhizal spores and propagules than undigested field soil (Reddell and Spain, 1991; Gange, 1993). The enrichment of spores and propagules in earthworm casts is thought to be due to the earthworms' selective feeding activity in the rhizosphere and/or in soil with a high organic matter content such as decaying roots where VA mycorrhiza are more abundant (Lee, 1985). It should be noted that the present study only showed the density of VA mycorrhizal spores and propagules in earthworm casts and field soil that were sampled once only. In order to assess the impact of this increase of VA mycorrhizal spores and propagules on soil fertility, further study is required on the population of earthworms, the quantification of earthworms' casting activity, and an assessment of the density of VA mycorrhiza in worm casts over longer periods.

Acknowledgements

MVR thanks the Rockefeller Foundation (New York) for financial assistance under an Environmental Research Fellowship in International Agriculture (Grant RF 89094#6).

References

Alexander, M., 1982. Most probable number method for microbial populations. In: A.L. Page, R.H. Miller and D.R. Keene (Editors), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Soil Science Society of America, Madison, WI, pp. 815–820.

An, Z.Q., Hendrix, J.W., Hershman, D.E. and Henson, G.T., 1990. Evaluation of the 'Most Probable Number' (MPN) and wet-sieving methods for determining soil-borne populations of endogenaceous mycorrhizal fungi. Mycologia, 82: 576–581.

Barea, J.M., 1991. Vesicular-arbuscular mycorrhiza as modifiers of soil fertility. In: B.A. Stewart (Editor), Advances in Soil Science, Vol. 15. Springer, New York, pp. 1–40.

Cochran, W.C. and Cox, G.M., 1959. Experimental Designs. 2nd edn. Wiley, New York.

Doube, B.M., Stephens, P.M., Davoren, C.W. and Ryder, M.H., 1994. Interaction between earthworms, beneficial soil organisms and root pathogens. Appl. Soil Ecol., 1: 3–10.

Fairchild, G.L. and Miller, M.H., 1988. Vesicular-arbuscular mycorrhizas and soil-disturbance-induced reduction of nutrient absorption in maize. II. Development of the effect. New Phytol., 110: 75–84.

Gange, A., 1993. Translocation of mycorrhizal fungi by earthworms during early succession. Soil Biol. Biochem., 25: 1021–1026.

Gerdemann, J.W. and Nicolson, T.H., 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet-sieving and decanting. Trans. Br. Mycol. Soc., 48: 235–244.

Harinikumar, K.M. and Bagyaraj, D.J., 1988. Effect of crop rotation on native vesicular–arbuscular mycorrhizal propagules in soil. Plant Soil, 110: 77–80.

Harinikumar, K.M. and Bagyaraj, D.J., 1994. Potential of earthworms, ants, millipedes and termites for dissemination of vesicular–arbuscular mycorrhizal fungi in soil. Biol. Fertil. Soils, 18: 115–118.

Lavelle, P., Rangel, P. and Kanyonyo, J., 1983. Intestinal mucus production by two species of tropical earthworms: *Millsonia lamtoiana* (Megascolecidae) and *Pontscolex corethrurus* (Glossoscoleciae). In: P. Lebrun, H. Andre', A. DeMedts, C. Gregoire-Wibo and G. Wanthy (Editors), New Trends in Soil Biology. Dieu-Brichart, Ottignies Louvain-la-Neure, pp. 405–410.

Lee, K.E., 1985. Earthworms: Their Ecology and Relationships with Soils and Land Use. Academic Press, Sydney, 411 pp.

Lee, K.K., Wani, S.P. and Vikram Reddy, M., 1995. Soil surface management and microorganisms. J. Soil Biol. Ecol., in press.

McIlveen, W.D. and Cole, H., 1976. Spore dispersal of endogonaceae by worms, ants, wasps and birds. Can. J. Bot., 54: 1486–1489.

Porter, W.M., 1979. The 'Most Probable Number' method for enumerating infective propagules of vesicular–arbuscular mycorrhizal fungi in soil. Aust. J. Soil Res., 17: 515–519.

Rabatin, S.C. and Stinner, B.R., 1988. Indirect effects of interactions between VAM fungi and soil-inhabiting invertebrates on plant processes. Agric. Ecosystems Environ., 24: 135–146.

Reddell, P. and Spain, A.V., 1991. Earthworms as vectors of viable propagules of mycorrhizal fungi. Soil Biol. Biochem., 23: 767–774.

Reddy, M.V., Ravindra, V., Balashouri, P., Kumar, V.P.K., Cogle, A.L., Yule, D.F. and Babu, M., 1995. Response of earthworm abundance and production of surface casts and their physico-chemical properties to soil management in relation to those of an undisturbed area on a semi-arid tropical Alfisol. Soil Biol. Biochem., in press.

Smith, G.D., Coughlan, K.J., Yule, D.F., Laryea, K.B., Srivastava, K.L., Thomas, N.P. and Cogle, A.L., 1992. Soil management options to reduce runoff and erosion on a hardsetting Alfisol in the semi-arid tropics. Soil Tillage Res., 25: 195–215.

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0929-1393/96/$15.00

Printed in The Netherlands

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