Soil Biol. Biochem. Vol. 6, pp. 235–239. Pergamon Press 1974. Printed in Great Britain.

RESPONSE OF SOIL TESTACEA TO SOIL MOISTURE FLUCTUATIONS

J. D. LOUSIER
Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4

(*Accepted* 14 *March* 1974)

Summary—Experiments illustrated that, as soil moisture increased in a Rocky Mountain aspen woodland soil-litter system, all species of Testacea recorded showed a significant increase in number of active individuals and a proportionate significant decrease in the number of cysts. The trends were less evident in the A_0L layer because of low variation in moisture content compared to the A_0F and A_0H layers. At higher soil moisture the larger species present (>60 μm) tended to retain a larger proportion of living individuals as cysts than did the smaller forms ($\leqslant 60$ μm) which numerically dominated the population.

INTRODUCTION

An abiotic factor that affects soil Protozoa directly and profoundly is soil moisture. Soil Protozoa are equipped with encystment and excystment mechanisms that are geared to the fluctuations in, and restrictions of, soil moisture. Soil Testacea also have evolved structural characteristics, e.g. globose tests, slit-like apertures and inner diaphragms in terricolous forms, which adapt them to various moisture regimes. Soil testacean populations are usually dominated numerically by only a few species (Stout, 1962; Bonnet, 1966), and these few species have small (<60 μm) or flattened tests (*Trinema*, *Euglypha*, *Corythion*, *Phryganella*, *Centropyxis* spp.), which are the most capable of living within the limits and changes of soil moisture (Stout and Heal, 1967).

Because of various morphological adaptations to the soil environment, and because of various microhabitat factors affecting testacean distribution, i.e. pore space, availability of water and thickness of water film, and availability of inorganic materials for test building, it should be expected that resistance against dryness and reactivation time after remoistening vary among different Testacea. Volz (1972) found that the reactivation time in Testacea depended on the duration of the period of dryness. He also found that other microfauna, i.e. ciliates, nematodes, rotatorians, tardigrades, responded at lower soil water contents than did the Testacea. Slow rates of reproduction (Heal (1964) found generation times of 8 days in culture and Lousier (1974) calculated generation times of 6–10 days in experimental field conditions), slower encystment and excystment mechanisms (Stout and Heal, 1967), and greater requirements in terms of water film thickness are responsible for the time lag in the response of Testacea to rising moisture conditions.

There is little direct evidence, however, of the effects of changes in moisture content within a soil on the numbers and species of Testacea. This paper describes the changes in numbers of individuals of particular species in response to a flutuating moisture regime in a well-drained aspen woodland soil in the Rocky Mountains of Canada.

METHODS

The site description, including climatic factors and soil profile description, and details of the methodology were given in Louiser (1974). Water was applied every 3 days at the rate of 3·78 l. per subplot (1 m^2). Four replicate slides were made from each layer of each sample according to the technique used by Coûteaux (1967)

Table 1. Variation in soil moisture levels (per cent wet wt) in watered and unwatered experimental plots, showing significant differences ($P = 0·05$)

	A_0L		A_0F		A_0H		Ah	
	Un	W	Un	W	Un	W	Un	W
17 Aug. 1970	20·7	22·7	21·2	22·9	34·4	36·2	13·6	11·6
4 Sept. 1970	12·0	35·9	19·1	54·4	29·9	57·9	12·1	27·5
22 Sept. 1970	15·2	28·7	30·4	60·8	41·8	63·7	17·5	26·4

Un, unwatered plots; W, watered plots.
Bracket denotes significant difference between values within the watered and unwatered plots (t-test).
Rule denotes significant difference between values in the watered plots and the values in the unwatered plots (t-test).

and each slide was examined entirely. The soil moisture contents, both control and experimental, are given in Table 1.

RESULTS

Two species (*Trinema enchelys*, *T. lineare*) comprised about 50 per cent of the living Testacea found, and these plus five others (*Euglypha laevis*, *E. rotunda*, *Centropyxis aerophyla*, *C. aerophyla* var. *sphagnicola* and *Phryganella hemisphaerica*) accounted for about 90–95 per cent of the total living Testacea. When soil moisture increased, the number of active individuals of each of these species tended to increase with a proportionate decrease in the number of encysted Testacea; when soil moisture decreased in the unwatered plots there was a decrease in numbers of active and encysted Testacea (Table 2a, b, c). However, the trends are much less marked in the A_0L layer than in the A_0F and A_0H (Fig. 1a, b, c).

Three families (Euplyphidae, Phryganellidae and Centropyxidae) comprised 95–100 per cent of the total living Testacea, but each family reacted differently to a decrease in soil moisture content. As the moisture content dropped in the unwatered plots, the Euglyphidae decreased in numbers of active and encysted forms, the Phryganellidae remained about the same and the Centropyxidae decrease (d) in numbers of active forms but retained the same number of cysts. However, as moisture contents increased in the water plots, the three families reacted similarly by having an increase in the number of active forms and a decrease in the number of cysts.

Table 2 illustrates also that as soil moisture contents increased in the watered plots, i.e. 17 Aug.–4 Sept. 1970, the proportion of active forms increased 2–3 times and the proportion of encysted forms decreased up to 8 times, with most of the active and encysted individuals belonging to the Family Euglyphidae. As shown by Table 3 it was evident that after the initiation of watering the species which exceeded 60 μm in size generally retained a higher proportion of cysts than did those species 60 μm or less in size. Also, the proportion of the active and the encysted population that was comprised of species with individuals $>60\ \mu$m decreased up to 6- and 19-fold respectively with depth in the profile.

DISCUSSION

The results of this study and the results given in Lousier (1974) have shown that adding large amounts of water to very dry field plots in a deciduous woodland stimulated a significant increase in numbers of individuals of testacean species. The good soil moisture conditions occurred when soil temperatures were at the annual maximum but no correlation existed between abundance changes and temperature (unpub-

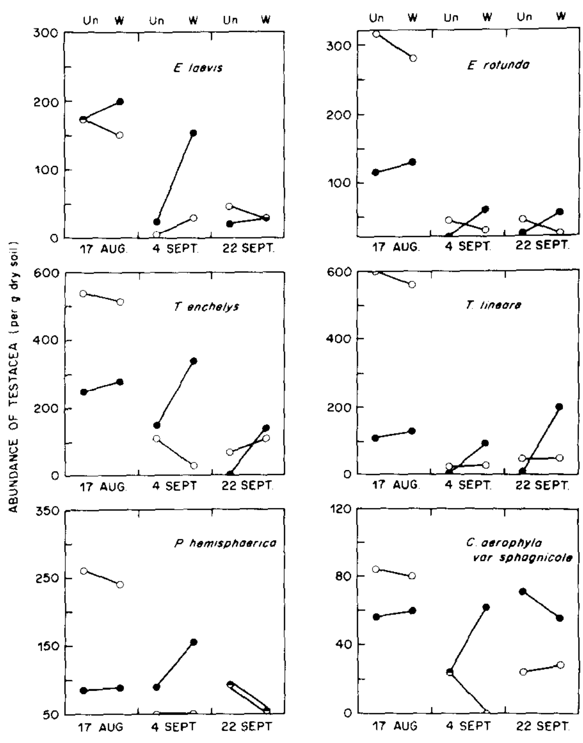

Fig. 1(a). Response of some species of soil Testacea to moisture changes in the A_0L layer (● active Testacea; ○ encysted Testacea) in August and September 1970.

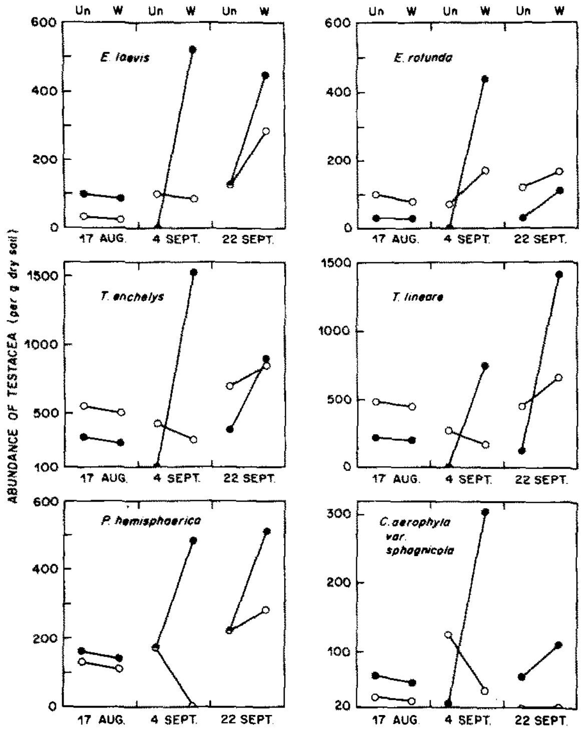

Fig. 1(b). Response of some species of soil Testacea to moisture changes in the A_0F layer (●—active Testacea; ○—encysted Testacea) in August and September 1970.

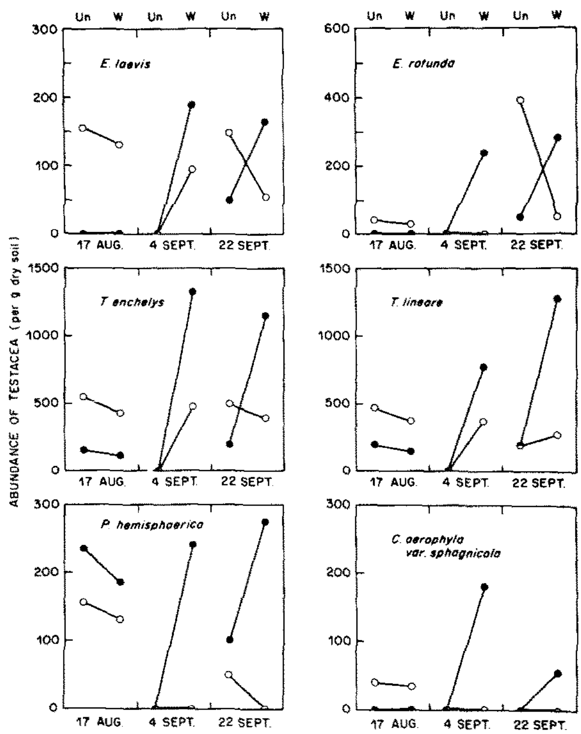

Fig. 1(c). Response of some species of soil Testacea to moisture changes in the A_0H layer (●—active Testacea; ○—encysted Testacea) in August and September 1970.

Table 3(a). Proportions of totals of active forms and encysted forms (expressed as %) per size category of testacea found in 1 g of dry aspen soil (Aug.–Sept. 1970)

Plot* treatment	Size (μm)	Horizon	Active forms 17 Aug.	Active forms 4 Sept.	Active forms 22 Sept.	Encysted forms 17 Aug.	Encysted forms 4 Sept.	Encysted forms 22 Sept.
Un	$\leqslant 60$	A_0L	85·0	83·2	59·0	87·9	30·1	35·9
	>60		15·0	16·8	41·0	12·1	69·9	64·1
Un	$\leqslant 60$	A_0F	73·6	100·0	86·1	82·1	76·4	88·2
	>60		26·4	0	13·9	17·9	23·6	11·8
Un	$\leqslant 60$	A_0H	100·0		100·0	95·4		96·5
	>60		0		0	4·6		3·5
W	$\leqslant 60$	A_0L	86·0	61·7	67·7	88·3	100·0	80·0
	>60		14·0	38·3	32·3	11·7	0	20·0
W	$\leqslant 60$	A_0F	73·9	94·0	92·9	82·5	90·0	91·4
	>60		26·1	6·0	7·1	17·5	10·0	8·6
W	$\leqslant 60$	A_0H	100·0	92·7	94·0	95·3	91·2	93·3
	>60		0	7·3	6·0	4·7	8·8	6·7

* Plot treatment: Un, unwatered plots; W, watered plots.

Table 3(b). Ratio of active forms to encysted forms (expressed as %) per size category of testacea found in 1 g of dry aspen soil (Aug.–Sept. 1970)

Plot* treatment	Size (μm)	Horizon	Active forms 17 Aug.	Active forms 4 Sept.	Active forms 22 Sept.	Encysted forms 17 Aug.	Encysted forms 4 Sept.	Encysted forms 22 Sept.
Un	$\leqslant 60$	A_0L	28·0	62·5	41·8	72·0	37·5	58·2
	>60		33·2	12·6	21·9	66·8	87·4	78·1
Un	$\leqslant 60$	A_0F	37·8	21·4	37·3	62·2	78·6	62·7
	>60		49·9	0	40·2	50·1	100·0	59·8
Un	$\leqslant 60$	A_0H	29·4		34·1	71·6		65·9
	>60		0		0	100·0		100·0
W	$\leqslant 60$	A_0L	31·8	85·4	63·6	69·2	14·6	36·4
	>60		36·4	100·0	76·9	63·6	0	23·1
W	$\leqslant 60$	A_0F	37·7	84·1	60·9	62·3	15·9	39·1
	>60		50·2	75·0	55·6	49·8	25·0	44·4
W	$\leqslant 60$	A_0H	27·9	75·3	81·8	72·1	24·7	18·2
	>60		0	72·3	80·0	100·0	28·7	20·0

* Plot treatment: Un, unwatered plots; W, watered plots.

lished data). The rapid increase in soil moisture was not indicative of the rate of moisture increase under natural conditions in the fall, but the moisture content reached in the watered plots was similar to that which occurred later in the fall. However, all species of Testacea showed a significant increase in number of active individuals and a proportionate significant decrease in the number of cysts as soil moisture increased. The trends were less evident in the A_0L layer, however, because of the low level of moisture variation (Table 1) compared to the A_0F and A_0H layers. The litter layer is much less compact than either the A_0F and A_0H layers and has poorer moisture retention capabilities.

After watering, the larger species present ($>60\,\mu$m) tended to retain a larger proportion of living individuals as cysts than did the smaller forms ($\leqslant 60\,\mu$m) which numerically dominated the population. It seemed likely that the micromorphology of the organic layers provided physical as well as physiological restrictions of soil water availability for Testacea. Griffin (1972) maintained that as soil water content fell below field capacity, the abundance and activity of soil bacteria and other micro-organisms decreased rapidly. It is probable that the applied increase in soil moisture selected against the larger species because moisture contents remained below field capacity, which seems to average between 70–85 per cent (wet weight) in the aspen woodland soil (unpublished data). Because of pore space requirements to accommodate the tests, those species larger than $60\,\mu$m may have adequate moisture conditions for very short periods during spring thaw and before autumn freezing when soil moisture may approach or exceed field capacity.

There is some conflict in the literature concerning the importance of soil moisture on protozoan abundance and distribution. The limited experimental evidence (Cutler *et al.*, 1922; Cutler and Dixon, 1927;

Sandon, 1927) supported the idea that soil moisture was not the principal controlling factor in protozoan abundance. Cutler *et al.* (1922) found no significant effect of soil moisture on protozoan numbers (two species of small amoebae and four species of flagellates) or encystment in their daily counts in their plots where the moisture content varied only from 12–23 per cent. Cutler and Dixon (1927), however, showed that reproduction of Protozoa fell when moisture content fell below 50 per cent water holding capacity (w.h.c.) of the soil. Of the four species studied only *Cercomonas crassicauda* was able to excyst and multiply at moisture contents below 17% w.h.c. and although excystment took place, there was little or no multiplication of any of the species at 33% w.h.c. Losina-Losinsky and Martinov (1930) found that protozoan activity depended upon the soil moisture and physical structure of the soil as well.

Other authors (Nikoljuk, 1963; Bonnet, 1964; Pussard, 1967; Stout and Heal, 1967; Bamforth, 1971) have stressed, empirically, the fundamental role of soil moisture in protozoan abundance, distribution and activity, and Lousier (1974) showed strong correlations ($r = 0{\cdot}873$) between fluctuations in soil moisture and variations in numbers of Testacea. When pedological parameters are added to the consideration of environmental factors affecting protozoan populations, such parameters as the base status of the soil, micromorphology of the profile, the nature of the organic cycle and the pedogenetic process involved must be assessed in conjunction with soil moisture in order to explain changes in population density and activity of protozoans. Interpreting seasonal fluctuations of soil Testacea requires measuring and evaluating such habitat characteristics as water holding capacity, pF relationships, chemical changes in the litter and humus, and habitat phenology.

Acknowledgements—I wish to thank Dr. J. B. Cragg for enabling me to work on soil testacean problems at the Environmental Sciences Centre (Kananaskis), and for his critical assessment of the manuscript. I extend appreciation also to Dr. O. W. Heal and Dr. G. Pritchard for their critical analysis of the paper. Financial support was provided by the National Research Council of Canada and the University of Calgary.

REFERENCES

Bamforth S. S. (1971) The numbers and proportions of Testacea and ciliates in litters and soils. *J. Protozool.* **18**, 24–28.

Bonnet L. (1964) Le peuplement thécamoebien des sols. *Rev. Écol. Biol. Sol* **1**, 123–408.

Bonnet L. (1966) Le peuplement thécamoebiens de quelques sols du Chili (I). *Protistologica* **2**, 113–139.

Coûteaux M.-M. (1967) Une technique d'observation des thécamoebiens du sol pour l'estimation de leur densité absolue. *Rev. Écol. Biol. Sol.* **4**, 593–596.

Cutler D. W., Crump L. M. and Sandon H. (1922). A quantitative investigation of the bacterial and protozoan population of the soil, with an account of the protozoan fauna. *Phil. Trans. R. Soc. B* **211**, 317–350.

Cutler D. W. and Dixon A. (1927) The effect of soil storage and water content on the protozoan population. *Ann. Appl. Biol.* **17**, 247–254.

Griffin D. M. (1972) *Ecology of Soil Fungi.* Chapman & Hall, London.

Heal O. W. (1964) The use of cultures for studying Testacea (Protozoa: Rhizopoda) in soil. *Pedobiologia* **4**, 1–7.

Losina-Losinsky L. and Martinov P. F. (1930) *Soil Sci.* **29**, 349–362. Quoted in Stout J. D. and Heal O. W. (1967), p. 174.

Lousier J. D. (1974) Effects of experimental soil moisture fluctuations on turnover rates of Testacea. *Soil Biol. Biochem.* **6**, 19–26.

Nikoljuk V. K. (1963) Bodenbewohnende Urtiere (Protozoa) und ihre biologische Bedeutung. *Pedobiologia* **3**, 259–273.

Pussard M. (1967) Les protozoaires du sol. *Ann. Épiphytes* **18**, 335–360.

Sandon H. (1927) *Composition and Distribution of the Protozoan Fauna of the Soil.* Oliver & Boyd, Edinburgh.

Stout J. D. (1962) An estimation of microfaunal populations in soils and forest litter. *J. Soil Sci.* **13**, 314–320.

Stout J. D. and Heal O. W. (1967) Protozoa. In *Soil Biology* (A. Burges and F. Raw, Eds), pp. 149–195. Academic Press, New York.

Volz P. (1972) Über Trokenresistenz und Wiederaufleben der Mikrofauna (insbesondere Protozoen) das Waldbodens. *Pedobiologia* **12**, 156–166.