The Bioscan 4 (3) : 535-538, 2009
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

IMPACT OF AGING OF EARTHWORM MIDDENS ON DENSITY, DYNAMISM AND BIOMASS OF MICROBIAL POPULATION

SURUCHI KUMARI, SITARA JABEEN, BHARTI SINGH RAIPAT[1] AND M.P.SINHA*
Department of Zoology, Ranchi University, Ranchi - 834 008
[1]Department of Zoology, St. Xavier's College, Ranchi - 834 001
E-mail: m_psinha@yahoo.com

KEY WORDS
Earthworm middens
Aging
Bacterial population
Bacterial biomass

Received on :
21.06.2009
Accepted on :
17.08.2009

***Corresponding author**

ABSTRACT

The impact of earthworm (*Lennogaster pusillus*,Stephenson) middens on microbial (bacterial) population and biomass has been studied in laboratory. A trend of increase in bacterial population density, its wet weight and biomass was recorded up to 21 days and thereafter a declining pattern was noticed. The initial bacterial population (number /g soil), wet weight and dry weight (mg/g soil) were found to be $21.9 \pm 1.096 \times 10^8$; $32.85 \pm 1.645 \times 10^{-4}$ and $6.57 \pm 0.329 \times 10^{-4}$ respectively while the peak values for the same population attributes were $27.7 \pm 1.250 \times 10^8$; $41.55 \pm 1.875 \times 10^{-4}$ and $8.31 \pm 0.375 \times 10^{-4}$ respectively on 21st day of observation. The change of quality of earthworm midden after aging has been discussed in the light of growth of bacterial population and biomass.

INTRODUCTION

Earthworm activity has been reported to be an important cause of spatial and temporal heterogeneity of soil properties in agro-ecosystems. Structures known as earthworm middens are formed at the soil surface by the feeding and casting activities of earthworms which contribute significantly to this heterogeneity. Earthworms play a major role in soil nutrient dynamics by altering the soil physical, chemical and biological properties. Their casts, burrows and associated middens constitute a very favourable microenvironment for microbial activity (Hale *et al*., 2005; Hale and Host, 2005)

By burrowing and leaving their casts, earthworms exert a great influence on the water infiltration and gas exchange in soils. The air porosity for the water-conducting pores made by earthworms in the silty loam subsoil has been reported as 0.328 and 0.168% at a depth of 0.24-0.34m and 0.34-0.44 m, respectively (Lipiec *et al*., 1998). Water infiltration in soils with earthworms is, commonly, several times faster than in soils lacking in earthworms (Lee and Foster, 1991;Piekarz and Lipiec, 1996). The quantity of earthworm middens left on the soil surface is between 2 and 50 t yr^{-1} depending on the type of soil and land use, whereas the total amount of earthworm middens on the surface, and below it, can exceed 1000 t yr^{-1} (Lee and Foster, 1991). Tomlin *et al*., (1995), in reviewing the subject, indicated that the stability of earthworm middens compared with bulk soil aggregates can be greater or similar depending on whether they are fresh or old. Thus the aging of earthworm midden is an important aspect which changes its properties not only the physico-chemical but also the biological. Assessment of microbiological (bacterial) population as its density, dynamics and biomass constitutes important aspects of biological properties which get affected by the process of aging. The stability of the casts is enhanced by bacteria in the soil increasing their secretion production of gums (McKenzie and Dexter, 1987; Haynesand Fraser, 1998) in passing through the gut or by the cementing effect of calcium (Lee and Foster, 1991). The increased stability of the midden and soil aggregates is the single most important soil property affecting soil erodibility (Horn *et al*., 1998; Reichert and Norton, 1994) through the influence of particle detachment due to water-drop impact, surface sealing and water infiltration. Earthworm middens are reported as the hot-spots of microbial activity and nutrient dynamics and represent a suitable model for studying earthworm-mediated influences on soil microbial communities by alteration of the patch structure of the microbial environment (Aira *et al*., 2009)

The microbial populations in earthworm midden have been studied (Piekarz and Lipiec, 2001; Groffman *et al*., 2004; Hortensia and Davis, 2009; Aira *et al*., 2009) but the literature pertaining to the impact of aging of midden on microbial population is very scanty. The present communication deals with the impact of aging (0 to 42 days) of earthworm midden on density, dynamics and biomass of bacterial population in experimental condition.

MATERIALS AND METHODS

The study was carried out in laboratory by culturing the earthworms (*Linnogaster pusillus*, Stephenson) in plastic trays. The middens were collected from the plastic trays with sterilized aluminum foil and used for further experiments. For studying the impact of aging the earthworm middens were kept in sterilized petri dishes covered by aluminum foil under normal room condition. The soil properties are presented in Table 1.

Dilution plate method (Waksman, 1922) was used for estimating the bacterial population in middens. The primary suspension of soil was prepared from 1 g soil and was diluted. For enumeration of bacterial population 1 mL inoculum was taken from aliquots of1:$10^7$and 1:10^8 dilutions of the primary suspension and Czapek Dox agar media was used for culturing the bacteria.

Table 1: Characteristics of soil taken for earthworm culture for collection of middens

Characteristics	Velue(M±SD; n=3)
pH	5.81 ± 0.07
Organic carbon (mg C g^{-1} soil)	6.52 ± 0.11
Nitrogen(mg N g^{-1} soil)	0.78 ± 0.01
Phosphorous(Kg P hec.$^{-1}$ soil)	27.9 ± 0.62
Potassium(Kg K hec.$^{-1}$ soil)	148.0 ± 0.49

Petri dishes were inoculated in incubator at temperature (28±2°C) for growth of bacterial colonies. After 24 hr, bacterial colonies were counted. For each experiment, three replicates of Petri dishes were incubated.

Bacterial population was calculated from the average number of bacterial colony determined by dilution plate method. This was based on the assumption that the number of bacterial colony per unit soil represents the number of bacterial cells. The mean fresh weight of a bacterium cell was taken as 1.5 x 10^{-12}g (Toth and Hammer, 1977). This value, when multiplied with the number of bacterial colony gave the fresh weight of bacteria. Assuming 80% of bacterial cell to be water (Clark and Paul, 1970) dry weight of bacterial biomass was calculated (Satpathy *et al.,* 1982). Student's t test was done to determine the significance of change in population and biomass.

RESULTS AND DISCUSSION

The bacterial population in midden in the beginning was 21.9±1.09 x 10^8 which gradually increased to 23.0±0.458 x10^8 and 25.5=0.450x10^8 reaching at its maxima as 27.7±0.650 x 10^8 on 7th, 14th and 21st day respectively. There after a sharp decline in bacterial population was observed. The change in population was found to be significant ($p<0.001$). The parentage increase in bacterial population over initial population was recorded as 1.1%, 16.43% and 26.48% on 7th, 14th and 21st day while the decrease was more pronounced as 30.59%, 52.51% and 76.25% over the initial population on 28th, 35th and 42nd day respectively (Table 2).

The wet weight (mg/g soil) of bacterial population increased by 5.02% (32.85±1.645 x10^{-4} to 34.5±0.687x10^{-4}) on 7th day (Table 2). On 14th and 21st day the increase was in order of 16.43 and 55.70%. After 21st day a sharp decline was recorded by 30.59%, 52.51% and 76.25% on 28th, 35th and 42nd day. A similar trend of rise and fall in biomass (mg/g soil) was observed. The biomass increased by 5.02% (on 7th day) 16.43% (on 14th day) 26.48% (on 21st day) and there after decreased by 11.59% (on 28th day) 52.51% (on 35th day) and 76.25% (on 42nd day).

The maximum biomass was recorded on 21st day as 8.31±0.375x10^{-4} mg/g soil which decreased to 1.56±0.75x10^{-4} mg/g soil on last day of observation.

Some physical properties and microbial activity of the casts of the earthworm *Aporrectodea caliginosa* have been investigated by Piekarz and Lipiec (2001) and compared with the properties of aggregates from the bulk soil of silty loam texture. The water stability of 20-day-old 8-9 mm aggregates from casts, as determined by the drop impact method, was significantly increased compared with those of 3-day-old casts and natural aggregates. The rate of wetting of the natural aggregates was substantially greater than that for the cast aggregates. The values of the crushing

Table 2: Bacterial population (number per g midden), wet. wt. and dry weight (biomass) as mg/g soil in different age of earthworm midden

Day of Obs.	Bacterial population in earthworm midden (M±SD)	Wet wt. of bacterial population in earthworm midden (M±SD)	Biomass of bacterial population in Earthworm midden (M±SD)
0	21.9±1.096 x 10^8	32.85±1.645 x10^{-4}	6.57±0.329 x10^{-4}
7	23.0±0.458 x 10^8 *(+1.10)	34.50±0.687 x10^{-4}*(+5.02)	6.90±0.137 x10^{-4}*(+5.02)
14	25.5±0.450 x 10^8*(+16.43)	38.25±0.676 x10^{-4}*(+16.43)	7.65±0.135 x10^{-4}*(+16.43)
21	27.7±1.250 x 10^8*(+26.48)	41.55±1.875 x10^{-4}*(+55.70)	8.31±0.375 x10^{-4}*(+26.48)
28	15.2±0.808 x 10^8*(-30.59)	22.80±1.212 x10^{-4}*(-30.59)	4.56±0.242 x10^{-4}*(-11.59)
35	10.4±o.556 x 10^8*(-52.51)	15.60±0.835 x10^{-4}*(-52.51)	3.12±0.167 x10^{-4}*(-52.51)
42	5.20±0.251 x 10^8*(-76.25)	07.80±0.377 x10^{-4}*(-76.25)	1.56±0.075 x10^{-4}*(-76.25)

Values in parenthesis are percentage increase (+) or decrease(-) over initial value; * = Changes produced are significant at 1% level; n = 3 strength of aggregates from casts and natural aggregates were not significantly different. The populations of bacteria, streptomyces and fungi in earthworm casts increased with the ageing of the casts. The increased water stability of cast deposits can be an important factor in reducing the high susceptibility to erosion.An increase in microbial population (bacterial) and biomass has been recorded with aging of the earthworm middens up to 21 days which is in agreement with the above finding.

The net effect of earthworms on the size of the soil microbial biomass has been a topic of some controversy in the literature. Several studies have shown that earthworms reduce microbial biomass, primarily by consumption, as soil passes through the earthworm gut (Wolters and Joergenson, 1992; Bohlen and Edwards, 1995; Devliegher and Verstraete, 1995; Zhang and Hendrix, 1995; Gorres and others 1997; Callaham and Hendrix 1998; Saetre 1998; Zhang and others 2000; Lachnicht and Hendrix, 2001). In contrast, other studies have found earthworm induced increases in microbial biomass (Parle, 1963; Shaw and Pawluk, 1986; Daniel and Anderson, 1992; Scholle and others, 1992; Tiwari and Mishra, 1993; Burtelow and others, 1998; Bohlen and others, 1999). Devliegher and Verstraete (1995) suggest that the net effect of earthworm on microbial biomass is a product of reductions in biomass during gut passage and stimulation due to mixing of organic matter into the soil profile. Brown and others (2000) emphasize the importance of temporal and spatial scale when evaluating the effects of earthworms on the soil profile, suggesting that fresh earthworm casts behave differently than aged casts and that earthworm effects are often restricted to specific areas in soil (the drilosphere).

The changed behaviour of fresh and old earthworm midden may primarily be due to variation in bacrerial population as the stability of midden increases with age atlest for three weeks due to product of secretion by bactrial population.

REFERENCES

Aira, M., McNamara, N., Piearce, T. and Dominguez, J. 2009. Microbial communities of lumbricus terrestris L. middens: structure, activity and changes through time in relation to earthworm presnce. *J. Soil and Sediments.* **9(1):** 54- 61

Bohlen, P.J. and Edwards, C. A. 1995. Earthworm effects on N dynamics and soil respiration in microcosma receiving organic and inorganic nutrients. *Soil Biol Biochem.* **27:** 341-8.

Bohlen, P. J., Parmelee, R. W., Allen M. F. and Ketterings, Q. M. 1999. Differential effects ofearthworms on nitrogen cycling from various nitrogen-15-labeled substrates. *Soil Sci. Soc. Am. J.* **63:** 882-890.

Brown, G. G., Barosis I. and Lavelle, P. 2000. regulation of soil organic matter dynamics andmicrobial activity in the drilosphere and the role of interactions with other edaphic functional domains. *Eur. J. Soil Biol.* **3-4:**177-198.

Burtelow, Ae. , Bohlen, P. J. and Groffman, P. M. 1998. Influence of exotic earthworm in vasion on soil organic matter, microbial biomass and denitrification potential in forest soils of the northeastern United States. *Appl. Soil Ecol.* **9:** 197-202.

Callaham, M. A., and Hendrix, P. F. 1998. Impact of earthworms (diplocardia: Megacolecidae) on cycling and uptack of nitrogen in coastal plain forest soils from northwest Florida. *USA. Appl Soil Ecol.* **9:**233-9.

Clark, F. E. and Paul, E. A. 1970. The microflora of grassland. *Adv. Agron.* **22:** 375-435.

Daniel, O. and Anderson, J. M. 1992. Microbial biomass and activity in contrasting soil matriala after passage through the gut of the earthworm *Lumbricus rubellus* Hoffmeister. *Soil Biol. Biochem.* **23:** 465-70.

Devliegher, W. and Verstraete, W. 1995. Lumbricus terrestris in a soil core experiment: nutrient enrichment processes (NEP) and gut-associated processes (GAP) and their effect on microbial biomass and activity. *Soil Biol Biochem.* **12:**1573-1580.

Edwards, W. M., Norton, L. D. and Redmond, C. E. 1988. Characterising macropores that affected porosity and surfacemicro-relief of soils. *Soil Sci. Soc. Am. J.* **43:** 851-856.

Gorres, J. H., Savin, M. C. and Amador, J.A. 1997. Dynamics of carbon and nitrogen mineralization, microbial biomass, and nematode abundance within and outside the burrow walls of anecic earthworms (*Lumbricus terrestris*). *Soil Sci.* **162:** 666–671.

Groffman, P. M., Patrick, J. Bohlen, Melany, Fisk, C. and Timothy, J. Fahey. 2004. Exotic earthworm invasion and Microbial biomass in temperate forest soils. *Ecosystems.* **7:** 45-54.

Hale, C. and Host, E. 2005. Assessing the impacts of European earthworm invasions in beech-maple hardwood and aspen-fir boreal forests of the western Great Lakes region. National park service Great Lakes Inventory and Monitoring Network Report GLKN/2005/11.

Hale, C. M., Frelich, L. E. Reich, P.B. and Pastor, J. 2005. Effects of European earthworm invasion on soil characteristics in Northern Hardwood forests of Minnesota, USA. *Ecosystem.* **8:** 911-927.

Haynes, R. J. and Fraser, P. M. 1998. A comparsion of aggregatestability and biological activity in earthworm casts anduningested soil as affected by amendment with wheat or lucernestraw. *Eur. J. Soil Sci.* **49:** 629-636.

Horn, R., Richards, B. G., Gräsle, W., Baumgartl, T. and Wiermann, C. 1998. Theoretical principles for modelling soilstrength and wheeling effects - a review-. *Z. Pflanzenähr. Bodenk.* **161:** 333-346.

Hortensia Brito-Vega and David Expinosa-Victoria. 2009. Bacterial Diversity in the digestive tract of earthworms (Oligochaeta). *J. Biological Sci.* **9(3):** 192-199.

Kretzschmar, A. 1987. The soil partitioning effect of theearthworm burrowing system. *Biol. Fertil. Soils.* **3:** 121-124.

Kooch, Y., Jalilvand, H., Bahmanyar, M. A. and Pormajidian,

M. R. 2008. Abundance, Biomass and Vertical Distribution of earthworms in Ecosystem units of Hornbeam forest. *Journal of Biol Sci.* **8:** 1033-1038.

Lachnicht, S. L. and Hendrix, P. F. 2001. Interaction of the earthworm *Diplocardia mississippiensis (Megascolecidae)* with microbial and nutrient dynamics in a subtropical Spodosol. *Soil Biol. Biochem.* **33:** 1411-1417.

Lee, K. E. and Foster, R. C. 1991. Soil fauna and soil structure. *Australian J. Soil Res.* **29:** 745-776.

McKenzie, B. M. and Dexter, A. R. 1987. Physical propertiesof the casts of the earthworm *Aporrectodea rosea*. *Biol. Fertil. Soils.* **5:** 152-157.

Parle, J. N. 1963. A microbiological study of earthworm casts. *J. Gen.* **31:** 13–22.

Piekarz, J. and Lipiec, J. 1996. Preliminary investigations onthe effect of soil bulk density and water content on earthwormactivity (in Polish). Zeszyty Naukowe, University of Agriculture, Cracow. **310(47):** 111-115.

Pickarz, J. and Lipiec. J. 2001. Selected physical properties and microbial activity of earthworm casts and non ingested soil aggregates. *Int. Agrophysics.* **15:**181-184.

Reichert, J. M. and Norton, D. L. 1994. Aggregate stabilityand rain-impacted sheet erosion of air-dried pre-wet clayeysurface soils under intense rain. *Soil Sci.* **158(3):** 159-169.

Saetre, P. 1998. Decomposition, Microbial community structure, and earthworm effects along a birch- spruce soil gradient. *Ecology.* **79:** 834-846.

Satpathy, B., Behera, N. and Dash, M.C. 1982. Microbial population, biomass and activity in some tropical soils of orissa, India. *Biol. Bull. India.* **4(3):** 150-157

Scholle, G., Wolters, V. and Joergensen, R.G. 1992. Effects of mesofauna exclusion on the microbial biomass in two model profiles. *Biol. Fertil. Soils.* **12:** 253-260.

Shaw, C. and Pawluk, S. 1986. Faecal microbiology of *Octolasion tyrtaeum*. *Aporrectodea turgida*, and *Lumbricus terrestris* and its relation to the carbon budgets of three artificial soils. *Pedobiologia.* **29:** 377–389.

Subler, S. and Kirsch, A. S. 1998. Spring dynamics of soil carbon, nitrogen and microbial activity in earthworm middens in a no-till cornfield. Springerlinks.

Tiwari, S. C. and Mishra, R. R. 1993. Fungal abundance and diversity in earthworm casts and in uningested soil. *Biol. Fertil. Soils.* **16:**131-134.

Tomlin, A. D., Shipitalo W. M., Edwards, W. M., and Protz, R. 1995. Earthworms and their influence on soil structure andinfiltration. In: Earthworm Ecology and Bio-geography inNorth America (Ed. P.F. Hendrix). Lewis Publishers. pp.159-183.

Toth, J. A. and Hammer, I. 1977. Quantitative microbiological studies on the soils of sikfokut project. *Acta, Biol. Debrecina.* **4:** 33-44.

Waksman, S. A. 1922. A method for counting the numbers of fungi in soil. *J. Bot.* **7:** 339- 341.

Wolters, V. and Joergensen, Rg. 1992. Microbial carbon turnover in beech forest soils worked by *Aporrectodea catiginosa* (Savigny) (Oligochaeta: Lumbricidae). *Soil Biol Biochem.* **24:** 171-177.

Zhang, B. G., Li, G. T., Shen, T. S., Wang, J. K. and Sun, Z. 2000. Changes in microbial biomass C, N, and P and enzyme activitiesin soil in soil incubated with the earthworms *Metaphire guillelmi* or *Eisenia fetida*. *Soil Biol. Biochem.* **32:** 2055–2062.

Zhang, Q. L., and Hendrix, P. F. 1995. Earthworm(*Lumbricus rubellus* and *Aporrectodea caliginosa*) effects on carbon flux in soil. *Soil Sci. Soc. Am. J.* **59:** 816–823.