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Microflora in Earthworm Burrow Walls

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ABSTRACT

Earthworms as ecosystem engineers play an important role in soil ecosystems. The effects of earthworm activity on soil processes differ between ecological categories and species creating distinctive microhabitats for soil microorganisms and other invertebrates. Due to their relatively large size and characteristic feeding behaviour, certain species have a significant impact on soil structure, soil fertility, plant growth and crop yield. This role is achieved inside or in the vicinity of burrows. Since one half of the nitrogenous waste of an earthworm is excreted through the body surface, it presumably accumulates in the burrow walls and affects the soil microbial community in those areas. Moreover, earthworm burrow walls harbour distinctive communities of soil animals like protozoa, nematodes and microarthropods, which presumably control microbial activity in these microhabitats. It is proposed that the most important effect of earthworms on soils may be the stimulations of microbial activity that occurs in casts. This may be the case also with burrows since not all earthworms cast at the soil surface; most species that deposit casts do so in their own burrows. The microorganisms associated with the burrow walls are species specific, different in composition and function and significantly different from soil only a few millimeters away. The present review emphasizes the interaction of microbes and earthworms and the significance of earthworm burrow wall as a 'hot spot' of microbial activity. It elaborates on the types of burrow walls and the microbes associated with it. Further research can shed light on the diversity of microbial flora in the burrow wall and surrounding soil.

Keywords: bacteria, burrow types, earthworms, fungi, microbial diversity

CONTENTS

INTRODUCTION

Soil an important factor that influences the productivity of our planet's ecosystems is vital for the existence of many forms of life and harbors a remarkable biodiversity. Being one of nature's most complex ecosystems soil contains thousands of different organisms, which interact and contribute to the global cycles that make all life possible. Soil forms an intricate network of communities, that again group themselves as smaller communities occupying specific microhabitats and microniches. The microhabitat of soil is affected in addition to physical and chemical factors by the micro and macro fauna and flora (Robert and Chenu 1992). The soil microflora, which shows varying degree of activity in different microhabitats are in turn influenced by macroorganisms. Bacteria, fungi and actinomycetes comprising of this microflora, are spatially distributed in soil.

An understanding of how these soil microorganisms work helps to know how ecosystems function. Significant modulations occur when the microorganisms are influenced by macroorganisms. Of the larger soil invertebrate groups, the most familiar are the earthworms which are the most significant and dominant. Earthworms affect soil physical structure by their tunneling activity which improves soil aeration, porosity, and permeability increase the moisture absorption and availability of moisture to plants. They produce plant growth stimulants and increase the mineralization of soil by mixing soil minerals with organic matter. The areas around the earthworm or the earthworm 'sphere' create a favorable microhabitat for soil microflora and stimulate microbial populations in the soil (Tiunov and Scheu 1999). It has been proposed that earthworms have a mutualistic relationship with microorganisms (Barois and Lavelle 1986; Trigo and Lavelle 1993; Lavelle *et al*. 1995; Kale and Karmegam 2010; Hong *et al.* 2011) and may contribute to the maintenance of soil fertility and soil microbial diversity. It is well recognized that earthworms are important to plant litter degradation and to exploit the organic resources available in the litter and soil, associate with microorganisms (Prakash *et al*. 2008). Gilbert White, a European naturalist, in the year 1789, postulated the importance of earthworm burrowing and feeding on the fertility of soil. In 1881, Charles Darwin was the first to actually document increased plant litter decomposition due to earthworm activity. Since Darwin's observations, many studies have been done on the role of earthworms in litter decomposition and fertility of

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soil. They are not essential to have in the soil, but their presence can be an indicator of good soil quality. Feeding, casting and burrowing activities of earthworms strongly modify soil structure, microbial community and dynamics of chemical processes (Lee 1985; Edwards and Bohlen 1996). Studies have shown that earthworm activity may contribute to the stabilization of soil organic carbon (Fonte *et al.* 2007), increase organic carbon and total N in the soil (Weihua and Yin 2007) and their burrows serve as ways of fresh C transport into deep soil horizons (Axel *et al.* 2008).The effects of earthworms on soil processes differ between ecological categories and species. Earthworm can affect soil microfloral populations either directly or indirectly *viz*. comminution, burrowing, casting, grazing or dispersal. There is a great deal of information suggesting that earthworm activity changes the microbial community structure of soil.

EARTHWORM BURROW WALL

Earthworms live mostly in horizontal burrows, selecting food from the soil, often feeding on organic materials that are on or just under the soil surface, deposit casts within their burrows or in other spaces within the soil. The burrows they create facilitate water and gas transport (Zhang and Schrader 1993) by mixing soil minerals with organic material (Hendriksen 1991). The excretion products of the earthworm are nitrogen rich and hence there is an increased level of nitrogen transformation in the area around the burrow walls. Since one half of the nitrogenous waste of an earthworm is excreted through the body surface, it presumably accumulates in the burrow walls and affects the soil microbial community in those areas.

Earthworm burrows are important in maintaining soil aeration, drainage and porosity (Tisdall 1978; Carter *et al.* 1982; Edwards and Lofty 1982a, 1982b). Burrows and burrow walls are surrounded by soil rich in nutrients and polysaccharides and are lined with protein rich mucus that gives the burrow walls and castings their consistency. These maintain the stability of the channels by binding soil particles together. In addition to mucus secretions nitrogen excretion from the earthworm bodies (mostly urea and ammonia) is also added to the burrow walls and/or to castings. The burrows can be significantly enriched with oxidized Fe (Jeanson 1971), plant available - P, N and exchangeable Ca and K (Graff 1967, 1971). The diameter of the burrows varies with the dimensions of the earthworms but is generally in the range of 1-10 mm, which places them among the largest of soil pores. Burrows enable earthworms to select conditions that suit them best from the range of microenvironments available in one or more soil horizons, while retaining access to forage for food at the surface at times when conditions are suitable. Burrow walls of earthworms with some minute variations in the physico-chemical characters from the main soil forms an important microhabitat for the habitation of microfloral community. By living in burrows earthworms are to some extent protected from diurnal and seasonal variability in the physico-chemical environment and from predatory pressure. The burrow wall that the earthworm creates in soil was called the 'Drilosphere' by Bouche (1975). He defined drilosphere as a zone 2 mm thick around earthworm burrow walls. Lavelle (1987) expanded its meaning to include all soil including microbial and invertebrate populations affected by earthworm activities. Hence drilosphere includes externally produced structures (middens, burrows, diapause chambers, surface and below ground casts), the earthworm surface in contact with the soil and the internal micro environment of the earthworm gut. It has been shown that earthworm drilosphere creates a favorable microhabitat for the soil microflora (Devliegher and Verstraete 1997; Tiunov *et al.* 1997; Tiunov and Scheu 1999). The importance of the drilosphere as a "hot spot" of microbial activity in the soil is now widely accepted, but little is known about its influence on the microbial community in earthworm burrows. Tropical endogenic earthworms seem to exploit soil organic resources by a mutualist earthworm/microflora digestion system (Lavelle *et al.* 1983). High microbial biomass and activity in earthworm burrow walls were recorded in a range of field and laboratory studies (Tiunov and Scheu 1999). Moreover, earthworm burrow walls and other zoogenous soil structures harbour distinctive communities of soil animals, e.g. protozoa, nematodes and microarthropods, which presumably control microbial activity in these microhabitats (Hamilton and Sillman 1989; Anderson and Bohlen 1998; Maraun *et al*. 1999; Tiunov and Kuznetsova 2000; Tiunov *et al*. 2001a).

TYPES OF BURROWS FORMED BY EARTHWORMS

Earthworms are grouped into 3 types based on their habitats and the types of burrows they form are different in each case (Lee 1985).

a) Burrows formed by anecic earthworms: These are formed by species that feed on surface litter and are more or less permanent refuges in underlying soil horizons. They are usually vertical for most of their length, sometimes branching near the top to several entrances. They may extend deep into the soil, even up to 3m or more and have smooth linings built up by compression of soils and mucus secreted by the earthworms, which probably help to maintain high humidity and reduce water loss.

b) Burrows formed by endogeic earthworms: These species feed on food in subsurface soil horizons. Their burrows are predominantly horizontal, but have some vertical components and some openings to the soil surface. They too extend deep into the soils and are smooth walled, with a surface layer usually thinner than that found in burrows of anecics. Many burrows are partly or wholly packed with casts or with soil from overlying horizons, carried down by water. The intensity of the burrowing activity of these species is related to the amount and quality of food available.

c) Burrows formed by epigeic earthworms: These are more or less vertical made by species of earthworms that live near the surface as they retreat to enter a resting state in deep soil horizons during dry or cold seasons, or return to surface horizons when conditions permit them to resume active life. These burrows generally lack distinct linings; they are apparently made quickly, are used only once, and are ephemeral. They often terminate in roughly spherical mucus-lined chambers where individual earthworms have taken refuge.

MICROFLORA OF THE EARTHWORM BURROW WALLS

Nowak (1975) proposed that the most important effect of earthworms on soils may be the stimulations of microbial activity that occurs in casts. This may be the case also with burrows since not all earthworms cast at the soil surface; most species that deposit casts do so in their own burrows.

The mucous secretions contain high concentrations of organic N and ammonium (Needham 1957) and may serve as a substrate for fungi and bacteria (Edwards and Fletcher 1988). Also, earthworm castings that are ejected in the burrow and subsequently pressed into the side of the burrow wall contain elevated amounts of nitrate and ammonium (Edwards and Lofty 1980). The abundant nutrient resources for the soil microflora in the burrow walls continue the priming effect of the earthworm gut, thereby increasing over a short period mineralization rates and plant nutrient bioavailability (Brown *et al*. 2000). However the same study shows that when the burrow wall begins to dry and stabilize with age, microbial activity decreases. Loquet *et al*. (1977) and Tiunov *et al*. (1997) reported much greater total microbial count in burrow walls than in the adjacent soil. Devliegher and Verstraete (1997) found an increase in total bacterial, but not fungal count in *Lumbricus terrestris* burrow linings in laboratory studies. Idowu *et al.* (2006) reported that the total aerobic and anaerobic counts of microflora were higher in casts than in surrounding soil. Specific microorganisms are also found to be more abundant in the drilosphere than bulk soil. There are fewer studies to support this claim though and very little information is available on the taxonomic composition of microbial communities in burrow walls.

Several studies have shown that numbers of bacteria and microbial activity are generally enhanced in the drilosphere as compared to bulk soil (Parle 1963b; Edwards and Fletcher 1988; Barois 1992; Daniel and Anderson 1992; Kristufek *et al*. 1992; Pedersen and Hendriksen 1993; Trigo and Lavelle 1993; Tiunov *et al*. 1997). Studies also suggest that the structure of the microbial communities within earthworm casts and burrows is different from that in the surrounding or bulk soil. Another study showed that nematodes, microbial respiration, and inorganic nitrogen were more abundant in the drilosphere when compared to bulk soil, and microbial biomass C was lower (Gorres *et al*. 1997). Polyanskaya and Tiunov (1996) showed that fungal hyphae were less dense and bacteria were more abundant in the drilosphere than bulk soil. Experiments with burrow walls of *Lumbricus terrestris* in forest soils show 2.5 times larger microbial biomass compared to surrounding soil (Tiunov and Dobrovolskaya 2002).

There have been contrasting effects on microbial biomass, with microbial biomass increasing, decreasing, or showing no net change relative to soil unaffected by earthworms (Brown 1995).The differences in microbial activity, numbers and species indirectly support the hypothesis that the bacterial community structures of these habitats are different from that of the surrounding soil. Hence it can be safely assumed that soil material associated with earthworm burrows may provide a substantially different environment to soil microflora thereby making it a significant area of study.

BACTERIA AND ACTINOMYCETES

Bacteria are important in soils because they contribute to the carbon cycle by fixation and decomposition. Some bacteria are important decomposers and actinomycetes are particularly effective at breaking down tough substances such as cellulose and chitin. Bacteria are significantly concentrated at the surface of the burrow walls and within the adjacent 2 mm of the surrounding soil. This microenvironment comprises less than 3% of the total soil volume but contains 5-25% of the whole soil microflora and is where some functional groups of bacteria predominate (Lavelle and Spain 2001).

Fifty five percent of the bacteria isolated from burrows were capable of fixing nitrogen and 16% of them were denitrifying with maximum populations at 20-40 cm depth in the soil (Bhatnagar 1975). Similarly, Parkin and Berry (1999) found elevated populations of nitrifying and denitrifying bacteria and increased rates of nitrification and denitrification in the drilosphere as compared to bulk soil. The excretion products that the earthworms secrete are nitrogen rich and are probably responsible for the elevated levels of nitrogen transformation in the drilosphere. Also, a higher number of actinomycetes and *Vibrio* spp. were found in earthworm casts as compared to bulk soil (Mariaglieti 1979; Contreras 1980). Karsten and Drake (1995) found more anaerobes and cellobiose-utilizers in earthworm guts than in soil. Burrow walls of *L. terrestris were* dominated by *Cellulomonas* and *Promicromonospora.* They also had *Azotobacter*, *Streptomyces*, Myxobacteriales, and motile Gram-negative rods (mostly *Aquaspirillum*, *Alcaligenes* and *Enterobacter*) whereas *Bacillus* and *Streptomyces* prevailed in the control soil.

Effects of earthworms on the microbial community depend, in part, on the timing of the measurement. Some effects of earthworms may become apparent only after an extended period of time because changes that affect microbial community composition and trophic interactions, such as diffusion of nutrients beyond the burrow walls and development of pore structure in the burrow walls, may occur gradually. Clegg *et al*. (1995) found that total bacterial counts in burrow and bulk soil were initially no different, but increased through time in casts and remained elevated compared with bulk soil. Also at greater depths the contrast between drilosphere and bulk soil and the relative contribution of burrow walls to the total soil microbial activity probably increase strongly (Stehouwer *et al*. 1993; Joergensen *et al*. 1998). Tiunov and Scheu (1999) in a study of three forest ecosystems in two seasons have shown that the volume of bacteria in the drilosphere increased in the lime forest soil (up to a factor of 5.5 in June, by a factor of 2.1 in October) and in the oak and beech forest soil by factors of 3.0 ± 3.2 and 2.5 ± 2.6, respectively. Large number of rod-shaped bacteria was observed in the lime forest in June which were less abundant in October. Also the bacterial volume in the beech forest decreased by about 70% from June to October. In casts of *Allolobophora terrestris*, as the casts aged, the number of actinomycetes and bacteria appeared to increase. Counts of cellulose decomposers were found to be more numerous in fresh casts than in soils. Chitinolytic and proteolytic bacteria tended to decrease as the casts aged (Parle 1963b).

In a study of bacteria from burrow wall soil of two species of earthworm *viz.*, *Pontoscolex corethrurus* and *Lampito mauritii* at different time intervals of 30 and 45 days, a total bacterial count of 49.62×10^6 CFU/g at 30 days and 27.36×10^6 CFU/g at 45 days was observed in *P. corethrurus* and 0.8×10^5 CFU/g at 30 days and 5.3×10^6 CFU/g at 45 days was observed in *L. mauritii* (Kavitha *et al.* unpublished data). In the same study actinomycetes count in *P. corethrurus* was found to be higher at 45 days interval (13.84×10^6 CFU/g) compared to 30 days interval (5.97×10^6 CFU/g). In burrow wall soils of *L. mauritii* both actionmycetes and bacterial count was higher in 45 days compared to 30 days. Compared to control an increase in the total bacterial count in the burrow wall soil was seen only in the 45 day trials of *L. mauritii* whereas the total actionmycete count showed an increase in all the samples except the 30 days burrow wall soil. It was also found that in *P. corethrurus* 75% of the bacteria were capable of producing cellulase and amylase. Their number increased in the 45 day samples in *P. corethrurus* whereas their percentage was lesser in the 30 day samples compared to control soil. The burrow wall soil of *L. mauritii* did not harbour any cellulase producing bacteria whereas 92% of the bacteria were capable of amylase production in the 45 days samples. This study indicated the species specificity in harbouring of microbial communities in the burrow walls.

FUNGI

Fungi are important for immobilizing or retaining nutrients in the soil. Gange (1993) has reported the importance of earthworms in both the reduction and dispersal of soil borne animal and plant fungal pathogens and the spread of beneficial groups like the mycorrhizal fungi. Generally, a substantial part of the fungal community in “lined” burrow walls were typical “litter” species, mainly *Trichoderma* and *Mucor* (Tiunov and Dobrovolskaya 2002). In a study of the fungal species of the two geophagous earthworms *P. corethrurus* and *L. mauritii* (Kavitha *et al.* unpublished data) 3 species of *Trichoderma* and 2 species of *Mucor* apart from four species of *Fusarium*, *Penicillium* and *Aspergillus* and two species of *Cladosporium* were isolated from the burrow wall soils. A total of 55 species of fungi was isolated from this study. Tiwari and Mishra (1993) isolated 27 species of fungi from earthworm casts.

A study of the burrow wall soil of *L. terrestris* in three forest ecosystems revealed, the fungal volume to be higher in the burrow walls of beech and oak forests, where as the same was not seen in case of the lime forest (Tiunov and Scheu 1999). A seasonal increase in the fungal volume with a significantly higher volume in June than in October was also observed by these authors. Parle (1963b) has shown that in older casts of *Allolobophora terrestris*, the numbers of yeasts and filamentous fungi increased. The percentage of yeast was found to increase in the burrow wall of *P. corethrurus* especially after 45 days by Kavitha *et al*. (unpublished data). McLean *et al*. (2006) has shown that the presence of earthworms decreased zygomycete species abundance probably due to disruption of fungal hyphae. Studies on *P.corethrurus* and *L. mauritii* also have shown a reduction of zygomycete species in the burrow wall soil. Invasive earthworms can negatively impact the fungal community in forests by changing nutrient cycling (Scheu and Parkinson 1994; Steinberg *et al*. 1997). A study in the Canadian Rocky Mountains, conducted by Scheu and Parkinson (1994), demonstrated that within eight weeks of introduction, earthworms reduced the fungal content of the soil from an initial 55% to between 30 and 40%. Fungi that are most drastically affected by the actions of invasive earthworms are fast-growing fungal decomposer species such as *Mucor*, *Trichoderma* and *Fusarium* (Visser 1985). *Fusarium chlamydosporium*, *F. pallidoroseum* and *F. oxysporum* were found to decrease in the burrow wall soil of *P. corethrurus* and *L. mauritii* in a study by Kavitha *et al.* (unpublished data) whereas in the same study *Alternaria alternata* increased in the burrow wall soil of both species of earthworms and species of *Aspergillus* reduced in percentage.

EARTHWORM-MICROBE INTERACTIONS – BENEFITS AND ADVERSITY

Earthworms, soil microbes and their interactions are of key importance to the healthy functioning of the soil. Microorganisms are found abundantly in habitats of every condition of temperature pH and moisture. The recognition of biosphere with unique micro and macro faunal interactions and nutrient dynamics emphasizes the versatility and importance of microbes in sustaining life. Bestowed with remarkable inherent physiological and functional diversity, microbes have found application in agriculture, industry, medicine and environment. Much better known and exploited microbial activities are augmentation, supplementation and recycling of plant nutrients, so vital to sustainable agriculture. They form easily manipulated sources of value-added products like drugs, therapeutic proteins, antibiotics, vaccines and diagnostic tools. Notwithstanding the existing knowledge of microbes and microbial processes, we are still at the base of microbial diversity, which needs to be explored, investigated and exploited (Bhavdish *et al.* 2005).

Microorganisms are able to perform any chemical transformation during the decomposition of organic materials but their activity is highly dependent on macroorganisms. To help digest soil organic matter and exploit the organic resources available in the litter and soil, earthworms have developed a mutualistic relationship with the soil microbiota (Barois and Lavelle 1986; Lavelle 1987; Trigo and Lavelle 1993). While earthworms use organic matter as their nutrient source, the microorganisms ingested along with these nutrient sources actually elaborate the enzymes that make the nutrients available for the worm's use (Edward and Lofty 1972; Lee 1985). The ingested microbial populations play a key role in earthworm nutrition by helping in the breakdown of organic matter, particularly the components that the earthworms cannot utilize in their natural state (Hornor and Mitchell 1981). Scientists interesting call it the 'Sleeping beauty paradox', the basis of which is that soil microbial communities (the 'Sleeping Beauties') have the ability to digest almost any organic substrate, yet are dormant most of the time because they need assimilable carbon but have a limited ability to move throughout the soil in order to reach these resources. Earthworms (the 'Prince Charming') secrete mucus, move within the soil and provide the suitable temperature, moisture and organic resources within their guts for microbes to be activated (Brown *et al*. 2000). Jenkinson (1966) called this the 'priming effect'. A conditional mutualism is observed between earthworms and soil microorganisms where both perform 'mutual exploitation for mutual gain' (Bronstein 1994), permitting a better utilization of the ingested soil organic resources.

The present review emphasizes on the interaction of microbes and earthworms and the significance of earthworm burrow wall as a 'hot spot' of microbial activity. Further research can throw light on the diversity of the microbial flora in the burrow wall and surrounding soil. One needs to further examine community differences between the burrow wall and the surrounding soil and much more interdisciplinary research is needed to assess the potential role of earthworms in regulating the diversity of microflora in soil systems and the potentially beneficial or harmful effects this regulation may have on ecosystem function and plant growth in different ecosystems. Agricultural science and its impact on agricultural and environmental policy, pharmaceuticals and many other areas could benefit from this expanding knowledge base.

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