Introduction

Microbial Diversity

Microbial diversity in soil

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 extent of the diversity of microorganisms in soil is seen to be critical to the maintenance of soil health and quality, as a wide range of microorganisms are involved in important soil functions. The microbial biomass in the soil is the driving force of most terrestrial ecosystems because it is this biomass that largely controls the rates of turnover and mineralization of organic substrates. Microorganisms decompose organic matter and transform mineral nutrients in aquatic and terrestrial ecosystems.

The term biodiversity has been defined in various ways. In microbial terms, it describes the number of different types (species) and their relative abundance in a given community in a given habitat. In molecular-ecological terms, it can be defined as the number and distribution of different sequence types present in the DNA extracted from the community in the habitat. However, the term “community structure” implies that information is included on the numbers of individuals of different recognizable taxa (Liesack et al., 1997).

With respect to microbial diversity, the number of types present and the evenness of their distribution are important. A habitat with a larger number of species is more diverse, whereas in communities with the same number of species, if the species is evenly distributed, it is more diverse than when it is unevenly distributed (Hedrick et al., 2000). Soil microbial communities are often difficult to fully characterize, mainly because of their immense phenotypic and genotypic diversity, heterogeneity, and crypticity. Bacteria
are the most abundant microorganisms in soil, attaining populations in excess of $10^9$ individuals/gram of soil and representing perhaps as many as $10^4$ to $10^6$ different species (Torsvik and Ovreas 2002). The actinomycete and fungi are the next most numerous microorganisms in soil, numbering $10^6 - 10^7$ and $10^4$ to $10^6$/ gram of soil. The assessment of microbial biodiversity has the potential to provide useful insight into the health and functioning of soil.

**Earthworms and their interactions with microbes in soil**

Gilbert White, and European naturalist, observed in his journals, published as ‘The Natural History of Selbourne’ (1789): “Worms seem to be the great promoters of vegetation by boring, perforating, and loosening the soil, and rendering it pervious to rains and the fibres of plants; by drawing straws and stalks of leaves and twigs into it; and most of all, by throwing up such infinite numbers of lumps of earth called worm-casts, which, being their excrement, is a fine manure for grain and grass.”

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). Microorganisms in soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as soil structure formation; decomposition of organic matter; toxin removal; and the cycling of carbon, nitrogen, phosphorus, and sulphur (Van Elsas and Trevors 1997). In addition, microorganisms play key roles in suppressing soil borne plant diseases, in promoting plant growth, and in changes in vegetation (Doran et al., 1996). 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. The physical, chemical and biological properties of the soil habitat and their interactions with resident community of soil microorganisms have significant impact on growth and activity of microorganisms. The smallest organisms, bacteria, actinomycetes, fungi and algae which are collectively referred to as the microflora are both numerous and diverse. Microorganisms are able to perform almost any chemical transformation during the decomposition of organic material but their activity is highly dependent on
macroorganisms which influence the environment at larger scales of time and space (Lavelle et al., 1995). Soil animals range in size from microscopic (microfauna) to earthworms and small mammals (macrofauna). Numbers alone may not provide a very good indication of the importance of microbial group in the soil. Understanding of how these soil microorganisms work helps to know how the 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 are natural invertebrates of agro ecosystem belonging to the Phylum Annelida, Class – Chaetopoda and Order Oligochaeta and dominant in the temperate and tropical soils. They are the first group of multicellular eucelomate invertebrates to have succeeded to inhabit terrestrial environment (Kale and Karmegam 2010). They are hermaphrodites, both male and female reproductive organs are present in every single earthworm but self-fertilization does not generally occur. Earthworms, as ecosystem engineers, play an important role in many soil ecosystems and are one of the numerous ranges of burrowing organisms, which improve soils (Lavelle 1997). Due to their relatively large size and characteristic feeding behaviour, certain species have significant impacts on soil structure, soil fertility, plant growth and crop yields.

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. The main processes affected by feeding, casting and burrowing activities of earthworms are microbial activity (Lavelle et al., 1983; Scheu 1990; Pashanasi et al., 1992), dynamics of organic matter (Martin et al., 1992; Lavelle and Martin 1992), dynamics of chemical processes (Edwards and Bohlen 1996), nutrient release (Sharpley and Syers 1976; Bouche et al., 1987; Scheu 1987), and physical properties such as aggregation or infiltration (Bostrom 1986). They produce plant growth stimulants and increase the mineralization of soil by mixing soil minerals with organic matter.
There is a great deal of information suggesting that earthworm activity changes the microbial community structure of soil. It has been proposed that earthworms have a mutualistic relationship with microorganisms (Barois and Lavelle 1986; Lavelle et al., 1995; Trigo and Lavelle 1993) and may contribute to the maintenance of soil fertility and soil microbial diversity.

**Earthworm as indicators of soil health**

Gilbert White as long ago as 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 in his book ‘The formation of Vegetable Mould through the action of Worms’. Since Darwin’s observations, the role of earthworms in litter decomposition and fertility of soil has been well studied. They are not essential to have in the soil, but their presence can be an indicator of good soil quality. 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.

Soils with low earthworm populations often have a layer of undecomposed litter on the surface, with a sharp demarcation from the underlying soil, which usually has a poor crumb structure. Their importance to plant litter decomposition and fertility of soil is well recognized. Fertile soils harbor bacteria, fungi, actinomycetes, protozoa, proturans, symphylids, pauropods, spring tails, pseudoscorpions, insects and their larvae, millipedes, centipedes, slugs, snails, snakes, rodents and the earthworms. These fauna along with the flora contribute to soil health. The presence of earthworms in soils is generally indicative of the presence of other faunal representatives contributing to soil fertility. It would therefore certainly be appropriate to call earthworms as indicators of soil health.

Earthworms are generally more active in moist soils and contribute to maintain soil fertility in at least three ways: (i) they build and maintain a soil structure based on resistant macro-aggregates; (ii) they release nutrients from the plant residues to the soil organic matter; and (iii) they physically protect humus inside compact globular casts (Lavelle et al., 1994). Earthworm activity creates structures, globular and granular casts,
and galleries that modify soil aggregation and porosity, as well as the communication between pores. Earthworms also have an influence on nitrogen mineralization, either directly or indirectly. Direct release of nitrogen may come from metabolic products (e.g., faecal pellets, urine, mucus) or dead earthworm tissues. Indirect release takes place through changes in physical properties of the soil, fragmentation of soil organic matter and interactions between the earthworms and the soil biota (Lee 1985).

In addition to the physical mixing of the soil by burrowing activities, soil enrichment is achieved by speeding up mineralization of organic matter 2-5 times by the earthworms.

- Earthworm ingestion causes an increase in surface area of the organic wastes
- Ingestion removes senescent bacterial colonies and stimulates new bacterial growth
- Nitrogenous excretions from the worms enrich the soil formed from organic wastes
- Earthworm burrowing enhances the oxygen penetration
- Mineral nutrients are released through enhanced microbial mineralization and
- Earthworm feeding increase’s the interaction among microflora, thereby improving the flow and exchange of nutrients.

Earthworms also eliminate pathogens in the waste using two mechanisms:
- their gut microflora outcompete the pathogens; and
- they produce bacteriostatic substances.

Several valuable compounds are also produced through the earthworm microflora interaction. These include vitamins such as B\textsubscript{12} and plant growth hormones such as gibberellins and IAA.

**Earthworm Sphere**

The area around the earthworm or the earthworm ‘sphere’ creates a favorable microhabitat for soil microflora and stimulates microbial populations in the soil (Tiunov and Scheu 1999). The burrow wall that the earthworm creates in soil which is directly or indirectly influenced by the earthworm burrowing activity has been called zoosphere (Joffe 1936), vernisphere (Hamilton and Dindal 1983) or drilosphere (Bouché 1975). Bouche 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). With the drilosphere constantly changing in space and time, its importance 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.

**Effects of earthworms on soil microorganisms**

Studies have shown that the microorganisms present in the gut of the earthworms were also present in the soil in which the worms lived and hence it was concluded that the earthworms are unlikely to possess an indigenous microfloral population (Parle 1963; Satchell 1967). Evidence show that the numbers of microorganisms in the guts will greatly increase compared to the surrounding soil. It is therefore accepted that the earthworm casts will contain more microorganisms than surrounding soil (Dawson 1948, Parle 1963). The casts therefore are the foci from which soil microorganisms can spread into the surrounding soil. The casts of *Lumbricus rubellus* and *Octolasium lacteum* showed proportionately more fungi, actinomycetes and cellulose decomposing and butyric acid forming bacteria than the surrounding soil (Koylovskaya and Zhdannikova 1961). Differences in microbial populations in earthworm casts and surrounding soil are due to either environmental changes within the gut of earthworms or due to the food material which provides a rich substrate, thereby increasing microfloral activity. Since earthworm casts are usually rich in nitrogenous compounds, the presence of earthworms may help stimulate microbial decomposition of organic matter in the soil. Barley and Jennings, (1959) estimated that half of the increased rate of litter decomposition was due to the earthworms and half to the increase in microbial activity induced by the worm’s excretory products.
**Types of Earthworms**

Three ecological categories of earthworms - epigeics, anecics and endogeics, have been described. Each of these creates earthworm spheres with differing characteristics. The anecics and endogeics are known as soil ecosystem engineers and their impact on soils is great and may influence properties and processes at the ecosystem level. The functional role of epigeics is primarily that of litter transformers, like other litter invertebrates (Lavelle 1997).

*Anecics (Greek for “up from the earth” or “out of the earth”)*

Anecics the dominant earthworms (in biomass) in many temperate region soils, (Lavelle 1983) are primarily vertically burrowing species. They feed on surface litter and more or less are permanent refuges in underlying soil horizons. They often produce characteristic surface features called “middens” which are circular “mound-shaped” region around a surface of the burrow’s opening which is a mixture of surface organic materials (principally leaves) and soil. These are thought to act as “external rumens,” where microbes and fauna attracted to this ‘hot-spot’ enhance decomposition of uningested litter and organic fragments in casts, probably due to fungal colonization of these substrates and then preferential feeding on these fungi (Cooke and Luxton 1980), which are then often reingested, (Bouché 1983). Unlike epigeics, anecics ingest significant proportions of soil ranging from 61% in *Lumbricus herculeus* (Bouché and Kretzschmar 1974) up to 90% in *Millsonia. lamtoiana* (Ka Kayondo 1984) depending on seasonal activity and litter quality and availability throughout the year. Litters of higher N content or litters colonized by particular fungi species are preferentially ingested (Abbott and Parker 1981; Cooke and Luxton 1980). When surface conditions are less favorable or when constructing their burrows, anecics ingest more soil. The feeding and casting habits of anecics may deeply influence soil characteristics up to >1m depth. The translocation of litter, mucus excretions, air penetration and selection of soil particles enrich the burrow walls with oxidized Fe, OM and plant available nutrients (N, P, K, Ca). Hence, even though this region covers a small part of the total soil volume, burrow walls have higher microbial activities (Stehouwer *et al.*, 1993) and populations of ammonifying,
denitrifying, free-living aerobic and anaerobic N-fixing, hemicellulolytic and pectinolytic bacteria than surrounding soil unprocessed by earthworms. Examples of Anecic earthworms include *Lumbricus terrestris*, *Apporetodea longa*.

**Epigeics (Greek for “upon the earth”)**

Epigeic species live in, consume, comminute and partially digest surface litter, rarely ingesting soil particles. Since soils are affected indirectly via changes in the litter, the effects of epigeic earthworms are not truly drilospheric. Their mode of litter processing in natural systems results in greater nutrient leaching into the soil and reduced immobilization by surface-dwelling fungi (Anderson *et al*., 1983; Spiers *et al*., 1986). Since epigeics feed purely on litter and generally have a short gut transit time they probably depend on a rapid response of gut microbes to aid in digestion. Epigeic earthworm guts preferentially stimulate some microorganisms, and reduce others leading to a relative dominance of microorganisms different to that found in uningested soils. For example, various *Vibrio* spp. and *Streptomyces lipmanii* were the dominant bacteria and actinomycetes in *Eisenia lucens* guts (Contreras 1980; Mariaglieti 1979) but found in low abundance in its surrounding. Examples include *Dendrobaena octaedra*, *D. attemsi*, *D. rubidus*, *Eiseniella tetraedra*, *Heliodrilus oculatus* and *Lumbricus rubellus*.

**Endogeics (Greek for “within the earth”)**

Endogeics are the most prevalent earthworms (in biomass) in most tropical environments (Lavelle 1983) often being the only group present, particularly in agroecosystems. Endogeics are geophagous earthworms that feed on subsurface soil horizons and on soil OM of different qualities. They produce surface and below-ground casts of two main types: globular (compact, large) and granular (loose, small). Endogeic casts, with generally more clay and frequently more OM than uningested soil, contain and release significant amounts of nutrients; e.g., fresh casts of *Pontoscolex corethrurus* may have 2-8 times more inorganic P (Lopez *et al*., 1993) and NH$_4^+$ (Barois *et al*., 1999) than uningested soil. This N may result from selective ingestion of richer soil portions, microbial mineralization, enteronephridial N excretions or asymbiotic N$_2$ fixation in the gut (Barois *et al*., 1987). Endogeic digestion appears to be primarily mutualistic, with
highly variable amounts of intestinal mucus being produced in the foreguts, depending on feeding groups and species (Lavelle et al., 1995). Gut microflora are also preferentially stimulated or reduced depending on earthworm and microbe species, soil environment, and food ingested (Brown 1995). Fungal hyphae, active protozoa, algae, myxomycetes and nematodes may be digested, while encysted or protected forms survive gut passage and then rapidly proliferate in casts (Brown 1995). Cell viability is often positively affected so that higher populations of many microorganisms are detected in casts than bulk soils when using plate counts (CFU’s) or other methods (Brown 1995). Examples include *Allolobophora chlorotica, Apporectodea caliginosa, A. icterica, A. rosea, Murchieona muldali, Pontoscolex corethrurus and Lampito mauritii.*

Lavelle (1981) further subdivided the endogeic earthworms into three sub-groups (polyhumic, mesohumic and oligohumic), depending on the quality of organic matter they ingest. On the spatio-temporal level, endogeics may have completely different effects on OM dynamics due to the diversity of interactions (enhancement, statis and reduction) between earthworms and microorganisms at various levels of the drilosphere. This derived spatial heterogeneity in the soil profile contributes to the maintenance of the high diversity of soil organisms and in turn affects biogeochemical cycle (Anderson and Domsch 1978; Anderson and Bohlen 1988; Beare et al., 1995).

(i) *At the short term (a few hours) and small scale* - in casts and guts, earthworms selectively ingest and comminute particularly larger fractions, but only assimilate low proportions of OM (2-18%), thus egesting large amounts of C in casts and enhancing microbial activity in both the gut and casts (Lavelle and Gilot 1994).

(ii) *At the intermediate scales* - several days to weeks in casts and burrows microbial activity and populations in casts and burrows increase and then decrease (Brown 1995) when available substrates are exhausted or the casts and burrows dry out, producing unfavorable environmental conditions. OM decomposition, particularly in casts, generally increases, releasing nutrients and CO2 (and other gases), but may then decrease due to physical protection in compact casts like that of *Millsonia anomala*, reducing C mineralization up to 30% (Martin et al., 1991).
At scales of years to decades - in the soil profile, the role of endogeics is determined by the effects at the former scales. It appears that they stimulate an overall acceleration of OM turnover, yet cause slight decreases in total stocks. In a 7-year experiment with continuous maize in Yurimaguas, Peru, \textit{P. corethrurus} inoculation resulted in a loss of 3.2 t ha\(^{-1}\) C higher than in uninoculated plots (Charpentier 1996).

**Earthworm species in India**

India harbors about 11.1\% of the global earthworm diversity particularly in the biodiversity ‘hot spots’ of the Western Ghats and Eastern Himalayas. The Indian earthworm fauna is predominantly composed of native species, which constitute about 89\% of total earthworm diversity in the country (Julka and Paliwal 2005). Julka \textit{et al.} (2009) have reported the presence of 418 species of earthworms in India, majority of which are endogeic or geophagous. Though most of the Indian forms have specific preference for natural habitats, a few are exotic and ubiquitous. Indian species have successfully colonized different agro ecosystems. Julka (2001) has divided five earthworm diversity zones in India, \textit{viz.}, mega diversity, high diversity, medium diversity, low diversity and poor diversity zones. Around 45 exotic peregrine forms have also been introduced into India (Gates 1972; Julka 1988). These mostly occur in disturbed habitats created by deforestation and intensive cultivation. Successful colonization of almost all agro-climatic zones in India by the exotic species is mainly due to their inherent ability to withstand disturbance and interference (Julka 1988).

**Earthworms in Bangalore**

In a study of areas around Bangalore city comprising of gardens, pastures and arable lands, Kale and Krishnamoorthy (1978) have shown the distribution of 6 species. \textit{Lampito mauritii} was found to be the most dominant being identified in 12 of the 17 areas studied, though when other species were present \textit{L. mauritii} was the least abundant (0.66\%). \textit{Pontoscolex corethrurus} was the next dominant species in Bangalore being found in 6 of the 17 areas studied. Other species found in Bangalore include \textit{Drawida}...
barwelli impertusa, Octochaetoides beatrix, Polypheretime elongata and Perionyx excavatus.

Description of the earthworm species used in the study

*Pontoscolex corethrurus* Muller 1857

Phylum - Annelida
Class- Chaetopoda
Order - Oligochaeta
Family- Glossoscolecidae

*Pontoscolex corethrurus* is a ubiquitous, exotic, endogenic earthworm distributed in almost all tropical regions of the world. *Pontoscolex corethrurus* has been transported by humans throughout the humid tropics from its origin in South America, centered in forests of the Guayanese plateau (González et al., 2006; Righi 1984). It is a polyhumic earthworm living in the subsurface soil up to a depth of 10 cm. Its ability to overcome dry conditions in the quivascent state and reproduce by parthenogenesis has enabled it to survive in varying habitats. It survives in any soil type from lateritic to alluvial soil and has a very wide range of moisture tolerance. *Pontoscolex corethrurus* can be found under several land-use systems. It prefers good vegetable cover and has high frequency of distribution in orchards, gardens, coastal and hilly regions of Karnataka (Kale 1997). It has a highly efficient digestion system, wide tolerance for human disturbance and exceptional demographic traits, allowing it to quickly colonize disturbed places from where native earthworms have been removed (Lavelle and Pashanasi 1989; Tapia-Coral 2004). As an endogenous pantropical earthworm it plays an important role in the assimilation of phosphorous and in the recycling of other nutrients. This species is an efficient decomposer of organic matter (Guerra and Asakawa 1981) and it is a common species in managed ecosystems or in areas subjected to some type of alteration (Lavelle and Pashanasi 1989).

*Pontoscolex corethrurus* reproduce only at temperatures between 23 °C and 27 °C, being of low activity in soils with low field capacity of water retention (pF 2.5) (Bernardes and Kiehl 1994). However, they have great capacity to adapt to soils of different pH values,
texture and organic matter content (Bernardes et al., 1997). They are normally 60-120mm long, 4mm in diameter and the segments 90-212 are unpigmented. Setae in the clitellar regions are more strongly ornamented. Due to their widespread distribution and ability to adjust to a variety of environments, the species has been the focus of many studies (Guerra and Asakawa 1981; Barois et al., 1987; Pashanasi et al., 1992, Lavelle et al., 1998; González and Zou 1999; Liu and Zou, 2002). Pontoscolex corethrurus has a short generation time of 3.5-4 months and produces one hatchling per cocoon (Barois et al., 1999) It has a mean production of 25-57.5 cocoons/adult/yr. The interactions between the microorganisms and P. corethrurus during the transit of the soil inside their gut are known (Barois and Lavelle 1986). The microflora of the soil is also favored by the presence of the earthworm, for it induces the increase of the microbial activity of the original soil (Guerra and Asakawa 1981). Gates (1972) noted that P. corethrurus were implicated in rendering a South Indian soil cloddy and unproductive. They can be identified by the characteristic calciferous glands and the globular casts they produce. These casts are of high stability and comprised of coalescent round or flattened subunits of soil.

Lampito mauritii Kinberg 1867
Phylum - Annelida
Class- Chaetopoda
Order -Oligochaeta
Family- Megascolecidiae

Lampito mauritii described as one of the great wanderers (Stephenson 1923), and also referred in old documents as Megascolex mauritii (Kinb.) occupies a unique position for its wide distribution in most parts of India. It is native peregrine or cosmopolitan, all over the coasts and islands of the Indian ocean. They are reported occurring in Kapurthala (Punjab) many areas of West Bengal, Gujarat, Maharashtra, Madhya Pradesh, South Rajasthan, Andhra Pradesh, Tamil Nadu, Goa, Kerala, Andaman Islands, Lakshdweep Island, etc. This is the only species which dominates the arable lands in the plains of Karnataka and has the highest frequency of distribution in the state (Kale 1997).
The ability of this top soil and litter species to withstand a wide range of temperature, soil moisture content, adaptability to coexist with a wide range of other species of earthworms, the wide choice of food niches explains its large distribution (Kale and Krishnamoothry 1981; Ismail et al., 1990). It has strong regenerative properties and is presumed to be bi-parental. This species will luminance in the dark (Blackmore 2000). The mean doubling time, with reference to density and biomass of *L. mauritii* in different organic inputs is 38.05 and 33.77 days (Ismail 1997).

Adult earthworms are about 10-20 cm long, 3-5 mm in width and weighs around 1-1.5g with 166-190 segments, colour is dark yellow with purplish tinge at anterior end. Dorsal surface is purplish or dark brown with no calciferous glands. Male pores are on papillae and female pores near each other in pairs on XIV segment and spermathecal pores in three pairs. They reproduce round the year except April to July. Clitellum makes its appearance around 60-65 days and fully develop between 80-85 days and is ring shaped spreading over to four body segments, on XIV to XVII. Cocoon is oval, flat ventrally, curved dorsally and about 4mm in length, 2.5 mm in width and 20-25mg in weight. Incubation period is around 24±3 days. Mean production of 1.3 cocoons/ worm/day with 100% Hatching success (Ramalingam 1997). The key characters are the presence of seminal vesicles in ix and xii, no web between terminal prongs of penial setae and granular casts they form. These casts are comprised of an accumulation of small, fragile, fine textured pellets, with little structural stability.

**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. 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 burrows they create facilitate water and gas transport (Zhang and Schrader 1993), by mixing soil minerals with organic material (Hendriksen 1991). Moreover water, gas and
solute transfer processes can be enhanced by the presence of earthworm burrows (Kretzschmar and Monestiez 1992). Therefore, observation of burrow systems is important to understand both (i) the actual role of earthworms in the soil ecosystem and (ii) the influence of these burrow systems on soil transfer properties and (iii) the difference in the microbial diversity compared to surrounding soil.

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 (Carter et al., 1982; Edwards and Lofty 1982; Tisdall 1978). 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 1-10mm, 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.

Tropical geophagous 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**

a) *Burrows formed by the anecic earthworms*- The burrows 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 of epigeic species*- 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.

c) *Burrows formed by the endogeic species*- Their burrows are predominantly horizontal, but have some vertical components and some openings to the soil surface. They 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.

**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 mucus 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 and 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 L. terrestris burrow linings in laboratory studies. 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 (Barois 1992; Daniel and Anderson 1992; Edwards and Fletcher 1988; Kristufek et al., 1992; Parle 1963b; Pedersen and Hendriksen 1993; Tiunov et al., 1997; Trigo and Lavelle 1993). Studies also suggest that the structure of the microbial communities within the drilosphere, i.e. 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 L.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). 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 and depth 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. The differences in microbial activity, numbers and species indirectly support the hypothesis that the microbial 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.
Pontoscolex corethrurus

Casts of *P. corethrurus*

*Lampito mauritii*

Casts of *L. mauritii*