

## An overview of the symbiotic interaction between ants, fungi and other living organisms in ant-hill soils

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### ABSTRACT

Ants are one of the most abundant insects on the earth. They influence soil properties through the construction of their nests due to their burrowing habit and their capability to change physical characteristics, of their hills. The food of ants is essentially cellulosic material and since the ants do not produce cellulose dissolving enzymes, digestion of cellulose is carried out with the aid of micro-fauna including fungi, bacteria and microarthropods which are associated with them. Ants are involved in a symbiotic relationship with fungi for the last 50 million years. Most of the workers have attributed that the symbiotic association has evolved to such a level that the ants cease to produce their own digestive enzymes as the fungus associated with them provides them with hydrolytic enzymes and easily assimilated nutrients. An ant-hill is tremendously important for studying mycodiversity because it is built by worker ants that carry tiny pieces of dirt, leaf debris, animal and agricultural wastes from a particular area and deposit them at the mouth of ant colony. Thus, fungal diversity of ant-hill represents the fungal diversity of a particular area.

**Key words:** Ants, ant-hill, soil properties, cellulose, symbiotic association.

### 1. Introduction

An ant-hill is a mound of earth formed by a colony of ants while digging or constructing their underground nest. According to Stone (1985), “an ant-hill is a pile of earth, sand, or clay or a composite of these and other materials that build up at the entrances of the subterranean dwellings of ant colonies”. Each colony consists of series of underground chambers, connected to each other and surface of earth by small tunnels. There are rooms for nurseries, for food storage and for mating. The colony is built and maintained by a large group of worker ants. During excavating process of colony, these ants carry tiny bits of dirt in their mandibles and deposit them near the exit of the colony to form an ant-hill. Different ant species create nests varying in structure and size due to the difference in their feeding strategies. From pedological point of view, ants can build two types of nests, that is, type I nest and type II nest (Paton et al., 1995). Type I nests are less conspicuous in the landscape, are crater-shaped, small in diameter and height, their soil material is simply deposited on the surface and are highly susceptible to erosion [Fig 1a]. Type II nests are larger, coherent epigeic nests, often cemented and sometimes covered by vegetation [Fig. 1b]. It is very persistent through time that may significantly affect the spatial heterogeneity of the soil surface. The ant-hill protects the nest opening from water inflow during rain and also enhances wind driven nest ventilation. The colonies are closed and reopened by ants with seasonal periodicity, which in turn leads to respective disappearance and reappearance of ant-hills (Cosarinsky and Rocas, 2007).

## 2.1 Diversity of ants forming hills/mounds

Ants are social insects of the Family Formicidae, belonging to Order Hymenoptera of the Class Insecta. They are found in all the continents except Antarctica and few remote islands like Greenland and Hawaiian island. Ants vary in colour, mostly red or black, but few are green and grey also. Out of the estimated total of 22,000 species of ants (Fernandez, 2003), about 600 species have been found in India. There are varieties of ants species that form ant-hill/mound in diverse habitats. *Solenopsis invicta*, the red imported fire ant, is a major pest in the southeastern United States; it sometimes reduces species diversity and abundance of native ants (Morrison, 2002). Tschinkel (1998), found *S. invicta* to be more common in disturbed habitats of northern Florida, where it displaced native ants. *S. carolinensis* by constructing ant-hills in their habitats. Another important species of ant, which forms ant-hills is *Dorymyrmex smithi*, that is found from North Dakota to eastern Colorado and New Mexico, east through Texas to North Carolina and Florida (Snelling, 1995). *D. smithi* forms large polycalic (several nests) colonies (Trager, 1988), and the workers are particularly aggressive. In Argentina, *Dorymyrmex* and *Forelius*, are common in highly disturbed sites (Bestelmeyer and Wiens, 1996). Similarly, two rarely sampled ant species viz., *Myrmecina americana* and *Pseudomyrmex pallidus*, have been collected only from the disturbed sites (Van Pelt, 1953).

Beside these there are various other ants that form ant-hills in diverse habitats. For example, in Michigan, *Pheidole bicarinata* form mound on the dunes of Lake Michigan (Talbot, 1946). Likewise, *Pogonomyrmex badius* prefers open, exposed locations, and will move its nest if it is shaded (Carlson and Gentry, 1973). In India, red ants belonging to the genus *Solenopsis* and black ants belonging to the genus *Lasius* are among the most common ant species of ant-hills. However, Gadagkar *et al.*, (1993), reported 32 genera of ants from different localities of western Ghats.

## 2.2 Diversity of other organisms found in ant-hills

Besides ants, a number of other organisms like bacteria, fungi, actinomycetes, microarthropod, centipedes and millipedes are found in ant nests (Sleptzovaa and Reznikovab, 2006). Kotova *et al.*, (2013) studied the bacterial complex associated with several species of ants, the inhabiting soil, and their ant-hills. They found that majority of ant-hills were dominated by *Bacillus* (more than 80%) while the anthill of *Formica* was dominated by the *Flavobacterium-Bacteroides-Cytophaga* group. In addition, actinomycetes were widespread in the anthills of *Formica* and *Lasius*. Numerous staphylococci (20%) were also found in the *L. flavus* ant-hills, but the main dominants of the bacterial community were *Streptomyces* bacteria (68.5%). In the ant-hills of *Tetramorium*, many *Bacteroides* (28%) were found. Actinomycetes belonging to the genus *Streptomyces* were detected in the bacterial complexes of all studied ants, except for *F. cunicularia*.

According to Pokarzhevskij (1981), the abundant bacteria, actinomycetes and fungi in ant-hill attract numerous small soil invertebrates, including springtails. In these ant-hills, the ants maintain relatively stable and quite specific microclimatic conditions (Horstmann and Schmid, 1986), which determine to a considerable extent the specific structure of a microarthropod community. The abundance and diversity of springtails increases as the nest grows and develops. The abundance of springtails in large old domes with relatively constant humidity may significantly exceed their abundance in the surrounding soil and litter. Recently Stoev and Gjonova (2005) reported a variety of Myriapods, which is a subphylum

of Arthropoda containing millipedes and centipedes from ant-hills of *Formica* sp., *Camponatus* sp. and *Myrmica* sp. in Bulgaria. These Myriapods dwelling in ant-hills include *Brachydesmus* sp., *Polyxenus legurus*, *Megaphyllum* sp. and *Lithobius microps*.

### 2.3 Effect of ants on soil properties

Ants thrive in most of the ecosystems and form 15-20% of the terrestrial animal biomass, which exceeds that of the vertebrates (Schultz, 2000). Ants influence soil properties through the construction of their nests due to their burrowing habit and their ability to change physical characteristics, such as infiltration, water retaining capability, etc., of their hills/mounds. There is considerable information available on the effects of ants on soil conditions like bulk density, organic matter content and porosity within the nest area (Cammeraat et al., 2002; Dashtban et al., 2009). Lowered bulk density and increased soil porosity within the mounds improve aeration, alter temperature gradient and modify soil pH (Dean et al., 1997). According to Shakesby *et al.* (2003), water infiltration rate in ant-hill soil and that of surrounding area is accelerated by ants, which form large macropores [biopores] and mix organic matter with mineral soil during nest building. According to Lobry de Bruyn and Conacher (1994), the cortex covering the ant-hills likely plays an important role in absorbing the impact of the rain drops and in improving the water infiltration inside the ant-hills. This impact of ants on water infiltration and erosion could be more important in agricultural soils, where heavy machinery and herbicide use have reduced soil porosity and organic matter (Cerdeira and Jurgensen, 2008).

Lavelle (1997) considered ants as “soil engineers”, that is, edaphic organisms that are able to directly or indirectly modulate the resources to other species through their mechanical activities. Research reveals that many ant nests are maintained as highly stable environment in terms of moisture, temperature, pH, porosity, organic matter and CO<sub>2</sub> concentration (Cammeraat ELH and Risch, 2008) and thus provide unique habitat for other organisms with many well documented trophic interactions (Boultan AM and Amberman KD, 2006). Sharma and Sumbali (2013) studied the correlation of fungal diversity with soil pH of ant-hills and found that with the increase in the alkalinity of the ant-hill soil, there is corresponding increase in number of fungal species and their respective colonies. Similar results have been obtained earlier by Sui and Sinden (1951), who reported increase in growth and cellulolytic activity of fungal species with an increase in the soil pH. Later, Yamanaka (2003), also reported that pH of 7 to 8 is optimum for the growth of saprotrophic fungi. According to Kristiansen and Amelung (2001), ant nests are associated with high levels of nutrients and organic matter. In addition, the amount of soil porosity in ant-hill soil is also significantly higher than the normal soil. Gorositoa *et al.* (2006) studied the morphological changes in *Camponotus punctulatus* ant-hills of different ages in north-eastern Argentina and found that the percentage of soil porosity observed in the ant-hills was 24.6% in comparison to 8.3% found in the surrounding soil. Dean *et al.* (1997), studied the ant-hills of *Lasius flavus*, *Lasius alienus* and *Formica rufibarbis* on the north-facing slopes of Halle and observed that ant-hill soil had higher sodium and potassium levels but lower phosphorus and nitrogen levels than the soil away from ant-hills. Moisture content was lower but soil pH was significantly higher in ant-hill soils. In addition to this, plant species associated with ant nests usually differ from species growing in adjacent area (Folgarait, 1998). According to Roger and Hartnett, (2001) the mean number of grass species on mounds was lower than the mean number of grass species off mounds.

## 2.4 Ant – fungus symbiotic association

Most of the ants are generalist predators, scavengers and indirect herbivores but few have evolved specialised ways of obtaining nutrition. The leaf cutter ants belonging to the genus *Atta* and *Acromyrmex* feed exclusively on fungi. In addition, all the known ant species of tribe Attine are engaged in mutualistic symbiosis with fungi (Solomon et al., 2004). The ants provide the fungi certain proteolytic enzymes, which are present in their faecal matter and are in turn used for compensating the metabolic deficiency of the fungi during their growth (Martin and Martin, 1970). The ants also defend their fungal partner by keeping them pathogen free. The mycobiont partners in turn also perform certain functions besides providing food and nutrition to the ants. For example, the fungi maintain high humidity, which is required by the ants and their metabolic heat creates air current that ventilate the nests (Luscher, 1961). Schultz *et al.* (2002) studied the ecology, specificity and symbiosis between the fungus growing ant *Cyphomyrmex* and the associated fungus in wet forest of Panama, Florida (United States).

From mycobial standpoint, the nature of ant food source, the associated foraging strategy and the nesting behaviour may play a role in determining the mycobial community structure associated with nests (Boots et al., 2011). Since these fungi are critical for the functioning of soils, particularly through their specific role in cycling of nutrients such as carbon and nitrogen (Torsvik and Ovreas, 2002), they are also likely to play key role in processes within the ant nests. Soil nutrient stocks in ant nests can be significantly different from uncolonised ant free soil [42]. This is due to the fact that organic matter decomposition is greatly dependent on mycobial community present in the nest, besides the substrate quality in the ant-hill and feeding strategies of the ants. Eisenhauer *et al.* (2010) recently reported a strong link between above ground vegetation diversity and below ground mycobial diversity. Therefore, altered above ground diversity via ant-mediated soil modification could in turn affect mycobial communities below ground. Friese and Allen (1993) demonstrated that bioturbation by ants can lead to fungal spore accumulation, thus indirectly affecting the abundance and frequency of fungi in their nests.

The food of ants like that of termites is essentially cellulosic material. As the ants do not produce cellulose dissolving enzymes, the digestion of cellulose is carried out with the aid of micro-fauna including fungi, which are associated with them. According to Villesen *et al.* (2004) the fungus growing ants of attine tribe have involved in a symbiotic relationship with fungi for the last 50 million years. Most of the workers have attributed that the symbiotic association has evolved to such a level that the ants cease to produce their own digestive enzymes as the fungus associated with them provides them with hydrolytic enzyme and easily assimilated nutrients (Boyd and Martin, 1975). Erthal *et al.* (2009) studied the hydrolytic enzyme profile of the fungus, *Leucoagaricus gongylophorus* detected in the ant nests and observed highest specific activity for chitinase [ $\beta$ -chitin trioside] in the soluble fraction followed by pectinase, cellulase, laminarinase,  $\alpha$  and  $\beta$  glucosidase,  $\alpha$ -galactosidase and cellulase. These hydrolytic enzymes produced by *L. gongylophorus* play a vital role in the symbiotic association by breaking down the plant material incorporated into the nest by ants, liberating nutrients to stimulate fungal growth and provide a constant nutrient source for the ants. In return, the ants maintain the garden free of hazardous contaminating micro-organisms (Currie and Stuart, (2001), prune the fungus to stimulate production of small hyphal swellings [gongylidia], which are rich in lipid and carbohydrate (Bass and Cherrett, 1996). These gongylidia in turn form clusters known as staphylae and the ants preferentially feed on these staphylae. Chewing the staphylae release their content and it has been

calculated by Bass and Cherrett (1996) that whole fungus may provide upto 9.0% of the respiratory energy required by the worker ants. The fungus may also provide the ants with a source of sterols, possibly used by the ants for cell membrane synthesis or hormone precursors (Ritter, 1990). Maurer (1992) analysed the rectal fluid and whole bodies of *Acromyrmex octospinosus* and found 4-desmethylsterols that were present in the fungus and in the leaves cut by the ants. According to Holldobler and Wilson (1990), the ants also fertilize the garden with their enzyme containing faeces. Therefore, ant-fungal symbiosis is highly established.

### 3. Cellulose degrading fungi in ant-hill soil

Survey of literature shows that a large number of cellulolytic enzymes are produced by soil fungi. The role of some species of *Acremonium*, *Chaetomium*, *Trichoderma*, *Penicillium*, *Phanerochaete*, *Fusarium* and *Aspergillus* in the cellulose degradation process of various environments has been well documented (Bhat and Bhat, 1997). Generally, fungi produce three major kinds of cellulolytic enzymes: endoglucanase, cellobiohydrolase and cellobiase (Klyosov, 1990). Virtually, all the fungi that have been reported so far as producers of cellulases are mesophilic in nature and are distributed in diverse habitats like soil, dead organic matter, plant and animal residue (Makut and Godiya, 2010). These fungi degrade organic matter aerobically in their natural habitat and thus catalyse cellulolysis. The result of cellulolysis is the conversion of organic substrate into inorganic form, which is made available once again to the living organisms. Therefore, in natural biodegradation processes of lignocellulosic material, fungal cellulase plays an important role. In addition, fungal cellulases have found novel industrial applications in areas such as protoplast production and fermentation of biomass into biofuels (Mandels et al., 1974), animal feed production (Ishikuro, 1993), production of fermentable sugars and ethanol (Oksonen et al., 2000), production of detergents and other chemicals (Olsson and Hagerdal, 1997), food processing (Penttila et al., 2001), textile production (Cavaco-Paulo and Guibitz, 2003), cellophane processing as well as biotransformation of cellulose containing waste to fermentable sugar (Van Wyk and Mohulatsi, 2003), pulp and paper processing involving de-inking fibre surfaces and improvement of pulp drainage (Suurnakki et al., 2004).

Not much information is available about the cellulolytic active fungi associated with ant-hill soils in India as there has not been much research conducted about their nature and structure so far. However, recently Sharma and Sumbali, (2013) recovered cellulolytic active strains of *Trichoderma viride*, *T. harzianum*, *Penicillium chrysogenum*, *P. griseofulvum*, *Trichothecium roseum*, *Aspergillus niger* and *Acremonium implicatum* from ant-hill soils of Jammu [J and K state]. Some information is also available in scattered form about these cellulolytic fungi from other countries of the world. In Brazil, Della Lucia *et al.* (1987) studied fungus garden associated with the ant genus *Acromyrmex subterranean*. Kader *et al.* (1999) carried out scientific expedition to Bario highlands [Malaysia] and found 9 fungal species, of which two isolates of *Trichoderma* and *Aspergillus* were discovered to be highly cellulolytic in comparison to others. Recently, Cerda and Jurgenson (2011) studied the fungal species associated with ant mounds of three species [*Formica rufibarbis*, *Messor barbarum* and *Lasius grandis*].

### 4. Degree of cellulolysis in ant-hill soil

Three hypotheses were proposed to account for the degree of cellulolytic ability of the enzymes produced by fungal symbionts associated with ants (Nagamoto et al., 2011). First hypothesis is that the degree of cellulolytic activity of fungi is high and capable of degrading

several plant polymers making them important energy source (Martin and Weber, 1969). Second hypothesis states that such degradation is very small and only permits symbiont hyphae to penetrate nutrient rich cytoplasm of plant (De Siqueira et al., 1998). Third hypothesis states that fungus is metabolically inept against these polymers (Abril and Bucher, 2002). The later two hypotheses are more recent than the first one but are based on *in vitro* or highly indirect evidence. In view of this, Nagamota *et al.* (2011) carried out experiments to evaluate the cellulose degrading capability of the fungus associated with leaf cutting ants by cultivating it on *Paspalum notatum*. Complete degradation of most of the abundant leaf cells was observed and therefore, this *in vivo* study corroborates Martin and Weber's (1969) hypothetical proposition that cellulose is highly degraded by fungal symbionts, which serves as an important energy source.



**Fig. 1** Types of ant- hills.  
**a.** Type 1- Small and crater shaped.  
**b.** Type 2- Large and epigeic.

## 5. Conclusion

Thus ant-hill is a unique niche in which a variety of living organisms like bacteria, actinomycetes, fungi and microarthropods inhabit and interact with each other. The ant-hill also modifies the physical, chemical and hydrological properties of surrounding soil which in turn is the result of dynamic mutualism operating in the ant-hill. So study of fungi associated with it becomes important. Moreover, the strongly cellulolytic strains present in the ant-hill soil can be used for the local management of solid wastes. In addition, these organisms can also be harnessed for the industrial production of enzyme cellulase that has utmost importance in textile, laundry, detergent, pulp and paper industries. We believe that this review article justifies the launching of more detailed investigations on the role of ant-hills and the associated ants in diverse environments and the possible use of fungi associated with these ant-hills in different kind of industries.

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