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Evaluation of ant nest microenvironment from Darjeeling Himalaya

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Ants, the potent ecosystem engineers are capable of managing its own ecosystems by influencing the physico-chemical features of soil and thereby controlling soil microbial community. Ants collected foods, food residues and excreta are the important sources of nutrients for those microorganisms in nest area. Present studies on ant nest soil samples collected from south-west Darjeeling Himalaya area revealed that microbial load of nest soil were remarkably higher in number compare to that of the surrounding area irrespective of ant genus. From twenty nests samples only three different ant genera, viz, *Solenopsis, Monomorium* and *Componotus* have been identified as the members of family Formicidae. The nest soils were of mostly neutral range pH, with high moisture content and appeared chemically rich in organic carbon, nitrogenous compounds and available phosphorus. As Darjeeling Himalayan soil faces a constant demand for nutrients specially for nitrogen and phosphorus, ant-left nest soil can meet that following proper agro-ecological management practices.

Key words: Ant-nest microbes, Darjeeling Himalayan soil, Himalayan ant

INTRODUCTION

Ants have versatile ecological habitats except the extreme polar regions. These foraging soil insects are well known for redistribution and recycling of soil nutrients and are called ancient ecosystem engineers (Wagner *et al.* 1997). Colony of ant generally consists of series of underground chambers, connected to each other and surface of earth by small tunnels. They collect and store organic food and release excreta with in specific sites of nest which greatly influence physical, chemical and biological properties of the nest soil ecosystem. Besides ants, a number of other organisms like bacteria, fungi, actinomycetes, microarthopods, centipedes and millipedes are found in ant nests (Sleptzovaa and Reznikovab, 2006). However, the nature of ant food source, the foraging strategy and the nesting behaviour may play role in determining the microbial community structure associated with nests (Boots *et al.* 2012). Thus, ant nest could be claimed as a rich microhabitat and biogeochemical hotspots with unique assemblage involving ants and microbes (Clay *et al.* 2013). High moisture content and concentrated oxidizable organic matter as nutrients favors growth of fungi referred as 'fungal garden', the food for ant larvae and most of the ants farm fungal cultivars are monocultures (Scott *et al.* 2010). Presence of actinobacteria, capable of antibiotic synthesis, probably to protect those farm fungi has also been reported (Haeder *et al,* 2009). The amount of soil porosity in ant-hill soil is significantly higher ompare to the nearby soil which facilitates abundant growth of saprophytic fungi and aerobic bacteria (Kotova *et al.* 2013) and nitrogen-fixing and phosphate solubilizing microbes (Echezona *et al.* 2012) and other decomposer organisms on the ant refuse dump (Fernandez *et al.* 2014).

There are many examples from the literature on studies of the distribution, diversity and role of ant nest microbes. To our knowledge no previous study has addressed such ant-associated organisms from

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Himalayan and sub-Himalayan grass land or terrain ecosystems. Darjeeling, a part of the Eastern Himalayan zone with semi-evergreen, temperate forests. Soil is mild to highly acidic, rocky or sandy with low moisture content. Out of 828 species of ants (Formicidae) found in India and the highest variation (382 species representing 65 genera) has been recorded from the state West Bengal (Bharti, 2016). But the myrmecofauna of Darjeeling Himalaya and their nest specific microbiome as well are still unexplored.

In this study, attempts have been made to answer the questions (1) What kind of ants are present in this site of interest? (2) Do the nest soil differ from its surroundings? (3) Is this soil useful for local agriculture in Darjeeling Himalaya?

MATERIALS AND METHODS

Study site and ants

This study was undertaken in the south west part of Darjeeling Himalaya (India, 27°13´ to 26°27´ N latitude and 87°59´ - 88°53´ E longitude) at elevation ranging 1250-1440 m and in total area approximately 100 km X 100 km (Fig.3). Nests were not abundant and mostly confined in three main sites *viz,* Soureni, Nagri and Dhajea non-tea garden grassland locality with the dominance of ant family Formicidae, *Componatus compressus* under sub family Formicinae*, Solenopsis* sp.and *Monomorium* sp. under subfamily Myrmicinae. Altogether twenty densely populated active nest soil samples from their centre and twenty corresponding non nest samples considered as control (1.5 m apart from the tentative centre of nest) were collected during the period of spring to pre-monsoon i.e. March to June, 2016 (availability of nests during monsoon to winter was found extremely rare). Samples, 1cm beneath the surface, were collected in autoclaved bottles using ethanol sterilized borer and stored at 4°C in the field. In laboratory samples were sieved to collect ants and soil part was immediately air dried for further physicochemical measurements. For microbiological study, fresh soils were used within 48h.

Physico-chemical measurements of soil

Soil moisture content was determined gravimetrically and pH was measured in soil: water mixture (1g in 10 ml). The texture of soil sample was determined on the basis of particle size analysis following the standard method of mechanical Sieve shaking. Total organic carbon of soil was estimated following acid digestion method of Walkley and Black (1934) using concentrated sulphuric acid. For estimation of total nitrogen the Kjeldahl method (Keeney and Nelson, 1982) and for phosphate ($NaHCO_3$ extractable P) method as described by Olsen and Sommers (1982) were followed.

Free amino acids in soil sample (sieved and air dried) were extracted with deionized H_2O at a soil:solution ratio of 1:5 (w:v). From this solution 5 and 15 ìl per spot were applied on the activated silica gel TLC plate. Plates were developed by butanol, glacial acetic acid and water, 12:3:5. After drying the visualization of spots was done using ninhydrin solution, 0.2% in n-butanol, w/v. Rf value for each spot was determined and compared with those of authentic samples (Hi media) and the reference values accordingly.

Soil microorganisms

Nest and non nest control (both in triplicate) soil samples were collected in sterile container for microbial analyses and were held for 48h maximum in a refrigerator at 4°C. Total number of viable microorganism (CFU) in soil and types (bacteria, actinobacteria and fungi) were determined on the basis of their growth and colony morphology on nutrient agar medium. Probable availability and number of amylase and cellulose, exoenzymes synthesizing organisms from the same sets of soil sample were determined using starch agar medium (Hi media) and using Gram's iodine solution and CMC agar medium (Hi Media) and 0.1% Congo red respectively. Nitrogen-free agar was used to isolate the microorganisms having tentative ability of nitrogen fixation. Microbial growth on Pikovskaya agar (Hi media) medium with clear halo zone determined the solubilization of tricalcium phosphate by them (Pal Saha *et al.* 2014). In each case of microbe's isolation serial dilution followed by spread plate technique and incubation for 72h at 30°C - 37°C (as per requirements) was done.

Data management and analysis

Experiments were conducted in triplicate for the values of soil parameters. The three experimental values were averaged to generate the data points for final analysis. We used t-test to compare pH level, percentage of moisture, total organic carbon, available nitrogen, available phosphorus, and average number of microbes between nest soil and non-nest soil samples for three different sites and combined samples. The Pearson's Correlation coefficient was used to measure the correlation between these parameters. All data management and analysis was done using Microsoft Excel and SAS 9.2.

RESULTS AND DISCUSSION

Soil physico-chemical and microbial parameters: nest soil vs. non nest control soil

In this study we compared physico-chemical (Table 1a) and microbiological (Table 1b) parameters of nest soils of three available ants genera, with those of respective non-nest control soils, collected from south-west Darjeeling during the period of spring to pre-monsoon, 2016. All of the nest soils were of almost neutral pH range, with high moisture content (>20%) compare to typical Himalayan acidic dry soil. In contrast to recorded pH of Himalayan soil (4.8-5.4), characteristic shifting of nest soil pH towards neutral range (6.2–7.2) might be explained by the presence of ant-derived substances (Frouz and Jilkova, 2008), food residues and cation contents, specially on nest margin (Jílková *et al.* 2011; Farji-Brener and Werenkraut, 2017). However, earlier findings of Frouz *et al.* (2003) established that ants' activity increased in pH in acidic soil and decreased in pH in basic soils. It was also found that the texture of nest soils was mostly loamy compare to sandy to loamy sand quality of non-nest areas as recorded following the data analyses of soil particle size measurement (data not shown). The relation between soil quality and moisture content had been noticed from each site which proved the greater infiltration capacity of nest soil (Wang *et al.* 2017). It was clear that regardless of the ant species, the deviation in nest soil texture was the result of forging activity of insects.

The rocky soil of Darjeeling Himalaya is mostly poor in lime, magnesium, phosphorus and nitrogen (Ray and Mukhopadhyay, 2012) like those of our experimental control soils. Nest soils appeared extraordinarily chemically rich in available organic carbon (41-108 times, p<0.0001), nitrogen (14-38 times, p<0.0001) and phosphorus (about 10 times, p<0.0001) compare to non-nest soil samples (Table 1b). The experimental data revealed that the average higher contents of organic carbon, nitrogen and phosphorus in nest microenvironment were independent of ant genus. Accumulation of organic matter in nest might be due to ant's food residue, carbon mineralization and input of ant secreted substances (Dauber *et al.* 2001) which depend on season and age of the nest (Wagner *et al.* 2004). The enhanced contents of nitrogen and phosphorus could also be the action of unique microbial assemblage which were rare or absent

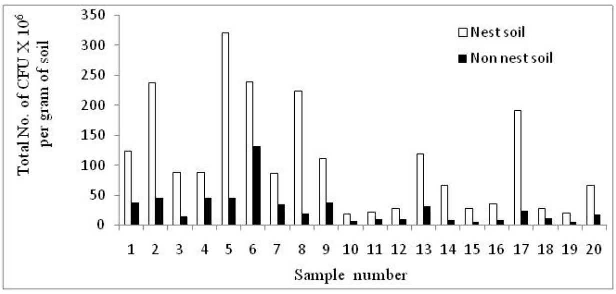

Fig.1: Total number of colony forming unit (CFU) per gram of soil of twenty different nest soil and non-nest soil samples was determined. Soil sample was diluted upto 10^6 fold followed by plating on nutrient agar medium and was incubated for 48h at 32°C

in respective non-nest soil. These findings corroborate with earlier researches (Boots *et al.* 2012).

The qualitative assay for free amino acids following thin layer chromatographic method showed positive for each of twenty nest soil samples in contrary of non-nest control soils where no as such free amino acids were found (Table 2). However, the type of amino acids in nest soil varied greatly.

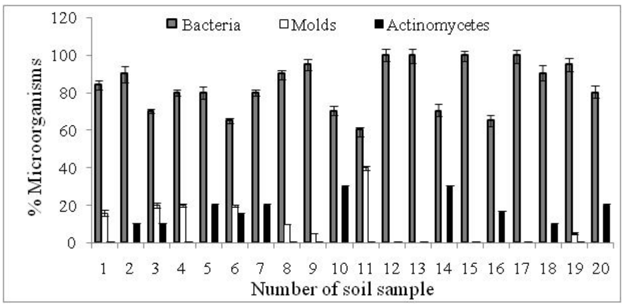

Fig.2: Total number of culturable bacteria, fungi and actinomycetes of nest soil and non-nest soil sample were determined. Results obtained on the basis of colony morphology and growth characteristics on nutrient agar plate and microscopic observation of individual colony grown on nutrient agar plate

The *Solenopsis* sp. ant nest soils mostly contained arginine followed by aspartic acid, cysteine and

Table 1a : Comparison of soil quality collected from of nest and non-nest area

Soil sample	Non-nest soil			Nest soil		
	Texture*	Moisture#, %	pH[a]	Texture	Moisture, %	pH
Dhajea						
Sample1	Loamy sand	13.4	4.8	Sandy loam	21.2	6.5
2	Loamy sand	11	5.0	Loam	23.5	6.5
3	Loamy sand	11.5	5.0	Loam	24.7	6.2
4	Loamy sand	13.5	5.4	Sandy loam	20.2	6.8
5	Loamy sand	12.2	4.6	Loam	28.6	6.5
6	Sand	7.5	4.8	Sandy loam	19.9	6.5
7	Sand	7.8	5.1	Sandy Loam	18.5	6.5
Nagari						
Sample 8	Sand	8.6	5.6	Sandy Loam	17.5	6.5
9	Sand	5.8	4.8	Loam	24.45	7.0
10	Sand	6.6	4.5	Sandy Loam	17.5	6.8
11	Sand	7.9	5.2	Loam	24.6	7.0
12	Sand	5.8	5.4	Sandy loam	18.64	7.0
13	Loamy sand	11.6	5.1	Sandy loam	18.5	7.2
14	Sand	9.5	4.6	Loam	27.5	7.0
Soureni						
Sample 15	Sand	7.5	4.8	Loam	27.6	7.0
16	Loamy sand	11.3	5.3	Sandy Loam	19.8	7.1
17	Sand	6.5	4.8	Loam	24.68	6.8
18	Sand	8.8	5.1	Loam	26.8	6.9
19	Sand	7.7	4.6	Loam	24.55	6.8
20	Sand	7.5	4.5	Loam	28.45	6.8

*Soil texture was determined on the basis of particle size analysis following the standard method of mechanical sieve shaking #Soil moisture content was determined following standard gravimetric method. [a]pH of the soil determined from the soil water suspension at $37^{o}C$

tryptophan where as *Monomorium* sp. nest soil samples appeared rich in lysine and phenylalanine and *Componotus* sp. with methionine followed by serine (Table 2). This specificity denoted that ants derived nitrogenous compound were the probable source of these free amino acids (Vele *et al.* 2010).

Experiments on the soil microbial load using nutrient agar media showed that in the nest area these were approximately 2-8 times higher (repeated measures ANOVA, p<0.0001) in colony forming units (CFU) per gram of soil than those of control non-nest soils (Fig. 1) and maximum (CFU 320.6 X 10^6/g) in soil sample No.5, *Componotus* nest, collected from Dhajea . It might be attributed by the availability of surplus nutrients, high moisture content and pH suitable for microbial growth. These findings were very similar to other ant nest reports (Golichenkov *et al.* 2011; Andersen *et al.* 2015) where microbial concentration were distinctly high compare to adjacent non nest area. On the basis of colony morphology and growth pattern on agar plates it was found that nest soil samples from all locations carried extensively high number of bacterial strains (60%-100%), mostly the gram positive bacilli type (data not shown) followed by fungal (0-40%) and actinobacterial isolates (0-30%) as depicted in Fig. 2. Thorough survey of microbial growth characteristics revealed that the nest area harboured limited variety of bacteria and fungi and unique actinobacterial strain, with high population density in individual nest system. The sites No. 3 and No.6 of Dhajea favoured the assem-

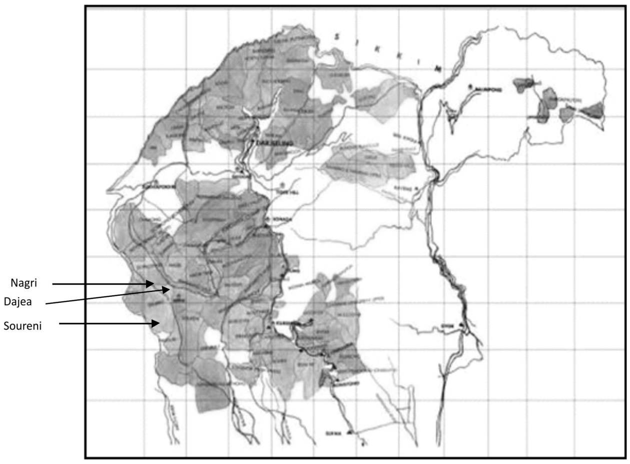

Fig.3: Darjeeling Himalaya within West Bengal and the three locations, Nagri, Dajea and Soureni from where sampling was done

Table 1b: Comparison of soil quality collected from of nest and non-nest area

Site	Variables	Number of Sample	Nest soil (Mean)	Number of Sample	Non Nest (Mean)	Mean difference	P value
Dhajea	PH	7	6.50	7	4.96	1.54	<0.0001
	Moisture %	7	22.37	7	10.99	11.39	<0.0001
	Total organic carbon %*	7	3.35	7	0.08	3.26	<0.0001
	Available nitrogen % #	7	1.53	7	0.04	1.49	<0.0001
	Available phosphorus % #	7	0.81	7	0.08	0.72	0.0006
	Average, No. of Microbes X 10^6/ gm of soil	7	168.90	7	50.18	118.70	0.0099
Nagari	PH	7	6.93	7	5.03	1.90	<0.0001
	Moisture %	7	21.24	7	7.97	13.27	<0.0001
	Total organic Carbon %*	7	3.47	7	0.04	3.44	<0.0001
	Available nitrogen % #	7	1.55	7	0.07	1.48	0.0006
	Available phosphorus % #	7	0.90	7	0.08	0.82	<0.0001
	Average, No. of Microbes X 10^6/ gm of soil	7	84.04	7	17.28	66.75	0.0363
Soureni	PH	6	6.93	6	4.85	2.08	<0.0001
	Moisture %	6	25.31	6	8.22	17.10	<0.0001
	Total organic Carbon %*	6	4.33	6	0.04	4.29	0.0008
	Available nitrogen % #	6	0.88	6	0.06	0.82	<0.0001
	Available phosphorus %#	6	0.90	6	0.09	0.81	<0.0001
	Average , No. of Microbes X 10^6/ gm of soil	6	61.10	6	11.42	49.68	0.0955
Total	PH	20	6.78	20	4.95	1.83	<0.0001
	Moisture %	20	22.86	20	9.10	13.76	<0.0001
	Total organic Carbon %*	20	3.69	20	0.05	3.63	<0.0001
	Available nitrogen %#	20	1.34	20	0.06	1.29	<0.0001
	Available phosphorus %#	20	0.87	20	0.08	0.78	<0.0001
	Average No. of Microbes X 10^6/ gm of soil	20	106.90	20	27.04	79.81	<0.0001

*Total organic carbon was estimated following acid digestion method of Walkley and Black (1934) #For estimation of total nitrogen and phosphate the nesslerization and ascorbic acid method were followed respectively as described by Jackson A. in 'Standard method for examination of water and waste water' by American Public Health Association, APHA (18th ed., 1992). Average number of soil microbes (CFU) estimated following the spread plate method on nutrient agar and growth for 72h at 32°C

blage of fungi-bacteria-actinobacteria where the soil pH ranges between 6.2-6.5, suitable for fungal growth and some unique bacteria and actinobacteria too as similar to other report (Sharma and Sumbali, 2013).

Biochemical characteristics of nest microorganisms

The individual isolate was tested for the synthesis of extracellular amylase, cellulase and nitrogenase and for solubilization of tricalcium phosphate following 48h solid plate cultures on starch agar medium, CMC agar medium, nitrogen-free Stockdale agar medium and Pikovskaya agar medium, respectively. About 10-100% total organisms from individual site appeared as either amylase or cellulase synthesizing organisms (Table 3) that means pre-monsoon nest soil microbiota resulted in very good decomposing activity, which contributed a considerable amount of easily available organic carbon to the soil and thereby to flourish microbial community. However, only eleven soil samples showed the presence of 3-14.66% free living nitrogen fixing bacteria compare to a large number of organisms secreting other three enzymes. 10-25% of the total isolated bacterial strains showed

Table 2: Free Amino acids available from different nest soil samples

Soil Sample / Ant genus	Amino Acid *									
	Acidic				Basic			Neutral		
	arg	asp	cys	glu	lys	met	phe	ser	thr	trp
Dhajea Sample										
1.*Solenopsis*	■	■								■
2.*Solenopsis*	■	■								
3.*Solenopsis*	■						■			■
4.*Monomorium*	■			■			■			
5.*Camponotus*						■				
6.*Monomorium*	■						■			
7.*Camponotus*						■				
Nagari Sample										
8.*Camponotus*						■				
9.*Solenopsis*	■									■
10.*Camponotus*						■		■		
11.*Monomorium*				■						
12.*Monomorium*					■					
13.*Solenopsis*	■				■				■	
14.*Solenopsis*	■		■							
Soureni Sample										
15.*Monomorium*					■		■			
16.*Monomorium*					■		■			
17.*Solenopsis*	■		■							
18.*Solenopsis*	■		■							
19.*Solenopsis*	■									
20.*Camponotus*						■		■		

□ absence of amino acid; ■ presence of amino acid;
***arg-arginine; asp-aspartic acid; cys-cysteine; glu-glutamic acid; lys-lysine; met-methionine; phe-phenylalanine; ser-serine; thr-threonine; trp-tryptophan
TLC of amino acids was performed on silica gel plate using solvent mixture -butanol, glacial acetic acid and water (12:3:5)

positive results for all four enzyme synthesis tests.

Interestingly, every nest horboured bacterial, actinobacterial or fungal strain, mostly a single type of microbial strain, which was efficient in mineralization of phosphate, contributing 33-100 % of total microflora for that site. Out of twenty, eleven nest soil samples facilitated the growth of free-living nitrogen fixing organisms up to the 15% of total isolates from that site. Limitation of the organisms under this category might be due to the presence of sufficient nitrogenous food residue or ant excreta. Wagner *et al*. (2004) showed the disproportionate distribution of N-space within nest site which depended on food habit of ants as well as excreta location in nest. Data derived from Table 3 did not reveal any correlation between the different types of microflora (bacteria, actinobacteria and fungi) isolated from nest of the specific ant and their tested biochemical features.

The reports on the activity of diazotrophs in fungal garden of leaf-cutter ants (Pinto-Tomás *et al.* 2009) or assemblage of fungi-bacteria-actinobacteria (Sharma and Sumbali, 2013) to increase the availability of free amino acids, total nitrogen and phosphorus approved the results using our ant nest microbial isolates. However, we agree the hypothesis of deposition of feed and excreta to increase in these nutrients (Vele *et al.* 2010).

Results of this study indicate that the ant-nest microenvironment is the naturally available nutrients rich bag. Indigenous ant-left-nest soil can be opted as it is better suited of plant growth in hill soil like that of Darjeeling. Furthermore, the enhanced bioavailability of nutrients could make the nest soil more approachable for hill agricultural use.

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Table 3: Number of organisms (%) isolated from twenty different nest soil samples showing ability to synthesize extracellular amylase, cellulase phosphatase and nitrogenase

	% nest organisms synthesizing enzymes*			
Nest soil sample	Amylase	Cellulase	Phosphatase	Nitrogenase
1	58.3±6.5	50±5.6	75±3.6	14±3.1
2	68.12±8.5	45.45±3.5	59.09±4	ND
3	75±5.5	37.5±3.5	62.5±4.6	ND
4	68±5.2	49±5.2	80±7.0	7.5±0.5
5	32±3.9	28±2.5	60±8.6	3±0.4
6	61±7.1	55±2.8	85±5.0	14.66±1.5
7	78±7.2	70±6.5	75±5.5	5.5±0.6
8	74.5±6.6	72±6.8	82.7±8.5	ND
9	44.5±4.8	40.5±2.5	76±6.5	ND
10	32±6.5	25±1.5	33.33±6.6	ND
11	10±2.2	18±5.5	90±9.8	7.5±0.5
12	25±1.6	10±1.2	80±6.8	3.5±0.5
13	50±5.0	35±3.5	60±7.7	4.8±0.4
14	60±6.3	50±6.8	50±8.5	ND
15	50±4.6	50±7.5	100±0	ND
16	33±2.5	30±5.2	100±0	10.5±1.2
17	45±4.6	45±7.4	77.77±3.5	6±0.4
18	100±0	100±0	100±0	5.5±0.4
19	100±0	80±5.5	88±5.5	ND
20	50±6.5	46±4.0	66.66±5.8	ND

*Results on the basis of microbial growth (positive/negative) on respective media, Starch agar, CMC agar, Pikovskaya agar and N_2–free Stockdale agar media. ND- Not detected

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REFERENCES

Andersen, S.B., Yek, S.H., Nash D.R. and Boomsma J.J. 2015. Interaction specificity between leaf-cutting ants and vertically transmitted Pseudonocardia bacteria. *BMC Evol. Biol.* **15**: 27-40.

Bharti, H., Guénard, B., Bharti, M. and Economo, E.P. 2016. An updated checklist of the ants of India with their specific distributions in Indian states (Hymenoptera, Formicidae). *Zoo Keys* **551**: 1–83.

Boots, B., Keithb ,A.M., Niechojd ,R., Breend, J., Schmidtb, O. and Clipsona, N. 2012. Unique soil microbial assemblages associated with grassland ant species with different nesting and foraging strategies. *Pedobiologia*, **55**: 33– 40.

Clay, N.A., Lucas, J. M., Kaspari M. and Kay, A. D. 2013. Manna from heaven: refuse from an arboreal ant links aboveground and belowground processes in a lowland tropical forest. *Ecosphere,* **4**:141.

Dauber, J., Schroeter, D., Wolters, V., 2001. Species specific effects of ants on microbial activity and N-availability in the soil of an old-field. *Eur. J. Soil Biol*. **37**: 259–261.

Echezona, B.C. Igwe, C.A. and Attama, L.A. 2012. Properties of Arboreal Ant and Ground-Termite Nests in relation to Their Nesting Sites and Location in a Tropical-Derived Savanna. *Psyche*, **2012**: 11.

Farji-Brener, A.G. and Werenkraut, V.J. 2017. The effects of ant nests on soil fertility and plant performance- A Meta Analysis. *J. Anim. Ecol*. **86**: 866-877.

Fernandez, A., Farji –Brener, A.G. and Satti, P. 2014. Moisture enhances the positive effect of leaf- cutting ant refuse dumps on soil biota activity. *Austral Ecol*, **39**: 198-203.

Frouz, J. and Jílková, V. 2008. The effect of ants on soil properties and processes (Hymenoptera: Formicidae). *Myrmecological News Vienna*, **11:** 191-199.

Frouz, J., Holec, M. and Kalcik, J. 2003. The effect of Lasius niger (Hymenoptera, Formicidae) ant nest on selected soil chemical properties. *Pedobiologia,* **47:** 205–212.

Golichenkova*,* M.V., Novoselovb, A.L., Marfeninaa,O.E., Dobrovol'skayaa, T.G., Zakalyukinaa, Yu.V. Lapyginaa, E.V. and Zamolodchikov, D.G. 2011, Microbiological Characteristic of Anthills of *Lasius niger. Biol. Bull.,* **38**: 277–282.

Haeder, S., Wirth, R., Herz, H. and Spiteller, D. 2009. Candicidin propducing *Streptomyces* support leaf-cutting ants to protect their fungus garden against the pathogenic fungus *Escovopsis. Proc Natl Acad Sci*. **106**:4742-4746.

Jílková,V., Matìjíèek, L. and Frouz, J. 2011. Changes in the pH and other soil chemical parameters in soil surrounding wood ant (*Formica polyctena*) nests. *Eur. J. Soil Biol.* **47**: 72-76.

Keeney, D.R. and Nelson, D.W. 1982. *Nitrogen in organic forms*. in Page *et al.* (Eds.) Methods of soil analysis. Part 2. Amer. Soc. Agron*.,* Madison, WI. pp. 643-698.

Kotova, A.A., Golichenkov, M.V., Umarov, M.M., Putyatina, T.S., Zenova, G.M. and Dobrovol'skaya, T.G, 2013. Microbiological activity in the anthills of fallow lands (Ryazan Region). *Pochvovedenie,* **2**: 31–34.

Olsen, S.R. and Sommers, L.E. 1982. Phosphorus. In Page *et al.,* (Eds) *Methods of soil analysis,. Part 2. Amer. Soc. Agron.,* Madison, WI pp. 403-430.

Pal Saha, S., Bhattacharyya, S. and Chakraborty, H. 2014. Solubilization of tricalcium phosphate by P(3HB) accumulating *Azotobacter chroococcum* MAL-201 *W. J. Microbiol. Biotechnol.* **30:** 1575-1582.

Pinto-Tomás' A.A., Anderson, M.A., Suen, G., Stevenson, D.M., Fiona, S. T. and Wallace, C.W. 2009. Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants *Science,* **326:** 1120-1123.

Ray, S.K. and Mukhapadhaya, D. 2012. A study on physicochemical properties of soil under different tea growing regions of West Bengal (India) *Int. J. Agri. Sci.* **4 :** 325-329.

Scott, J.J, Budsberg, K.J., Suen, G., Wixon, D.L., Balser, T.C. and Currie C.R. 2010. Microbial community structure of leaf cutter ant fungus gardens and refuse dumps. *Plos one*, **5**: e9922.

Sharma, V. and Sumbali, G. 2013, An overview of the symbiotic interaction between ants, fungi and other living organisms in ant-hill soils *Int. J. Environ, Sci.,* **4:** 432-443.

Sleptzovaa, E.V. and Reznikovab, Z.I. 2006. Formation of Springtail (Collembola) communities during colonization of ant-hills. *Entomol. Rev.*, **86:** 373–382.

Véle, A., Frouz, J., Holuša, J, and Kalèík, J. 2010. Chemical properties of forest soils as affected by nests of *Myrmica ruginodis* (Formicidae). *Biologia* **65:** 122—127.

Wagner, D., Brown, M.J.F. and Gordon, D.M. 1997. Harvester ant nests, soil biota and soil chemistry. *Oecologia* **112**: 232–236.

Wagner, D., Jones, J.B. and Gordon, D.M. 2004. Development of harvester ant colonies alters soil chemistry. *Soil Biol. Biochem.,* **36**: 797–804.

Walkley, A. and Black, T. A. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **37**: 29-37.

Wang, C., Wang, G., Wu, P., Rafique, R., Zi ,H., Li, X. and Luo, Y. 2017, Effects of ant mounds on the plants and soil microbial community in an alpine meadow of Quinghai- Tibet plateau. *Land Degrad. Develop*. **28** : 1538-1548.