ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 01, pp.119-128, January, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

Soil Microbial Diversity and its Biochemical Properties at two different Forest Stands of Meghalaya

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ARTICLE INFO

Article History:
Received 14th October, 2012
Received in revised form
06th November, 2012
Accepted 22th December, 2012
Published online 16th January, 2013

Key words:
Altitudes,
Biochemical properties,
Forest stands,
Soil microbial populations,
Physico-chemical properties.

ABSTRACT

Microbial diversity (fungi and bacteria), biomass carbon and nitrogen, respiration and physico – chemical properties of soil were studied for two years i.e., 2009 and 2010. The study sites selected were two different broad leaved forests of Meghalaya, (i) at Upper Shillong (1861msl) and (ii) at Mawkyrdep (889 msl). Results showed that CFUs of fungi and bacteria, microbial biomass carbon and nitrogen, respiration and moisture content, organic carbon, total nitrogen and available phosphorus except exchangeable potassium were higher in the soil at high altitude forest than at low altitude forest. Qualitatively, there was not much difference in the composition of the fungal flora at both the study sites. Majority of the fungal species isolated belonged to deuteromycotina. Species of *Aspergillus, Fusarium* and *Penicillium* were found to be dominant at both the study sites. The results also showed that all these parameters decreased with increase in soil depth. CFUs of fungi and bacteria showed significant positive correlations with microbial biomass carbon and nitrogen, moisture content, organic carbon, available phosphorus, total nitrogen. One way analysis of variance (ANOVA) showed significant variations ($p \leq 0.05$) between various parameters studied and the soil depths. Shannon diversity index and Simpson dominance index of fungi were highest at the surface and sub surface soil layer respectively.

INTRODUCTION

Soil microbial communities are widely recognized as an integrative component of soil quality because of their crucial involvement in many ecosystem processes (Warcup and Waksman, 1950). Microbes are highly versatile; they can carry out almost all known biological reactions. 80–90% of the processes in soil are reactions mediated by microbes (Coleman and Crossley, 1996; Nannipieri, 1994). They are essential components of the biotic community in natural forests, and are largely responsible for ecosystem functioning because they participate in most nutrient transformations (Hackl *et al*.,2004). Because microbial activities integrate soil physical and chemical properties, and respond to anthropogenic activities, microbiological properties of soil may be considered suitable biological indicators of soil quality (Lin *et al*., 2004). Several studies reported that the composition of the soil microbial community can be altered by plant species, plant diversity, vegetation or forest type (Mc Culley and Burke, 2004; Waldrop, 2000; Porazinska, 2003; Balser, 2005; Bartelt-Ryser, 2005), soil type, seasonal variability in water, temperature and availability of organic substances.

Although, soil micro organisms probably represent the world's greatest reservoir of biological diversity (Zhou, 2003) only 80,000 species have been described to date (Schmit, 2007) out of the estimated global fungal diversity which vary from 0.7 to 9.9 million species (Hawksworth, 1999, 2004). The number of unexplored habitats, which have proven to be rich in specialized and unique fungi, is still enormous (Suryanarayanan, 2005). Soil microbes mainly bacteria, fungi are concerned with all the biochemical processes which occur in soils and they play a vital role in maintaining soil productivity. It has been generally hypothesized that reduction in soil microbial diversity will result in reduction in the functional capability of soil (Giller, 1997). Although, the extend of microbial diversity is not yet known, microbial diversity indices can function as bio- indicator to show community stability and describing the ecological dynamic of community (Atlas, 1984) and analysis of soil microbial diversity is important to evaluate the importance of perturbations in soil systems (Turco, 1994). It can also provide an early indication of changes in soil long before it can be measured by changes in organic matter (Powlson, 1987). Microbial population size and community structure are sensitive to changes in chemical properties of the surrounding soil (Hodges, 1999; Schinner, 1996). Further, considerable evidence indicates that changes in the composition of a microbial community can be used to predict and dictate alteration in soil quality (Van Bruggen, 2000; Breure, 2005). Research on microbial diversity provides a basis for estimating the functional role of fungi in ecosystems. Recognition of the importance of soil microorganisms has led to increased interest in measuring the nutrients held in their biomass.

The importance of the SMB in soil functioning is well recognized. Soil microbial biomass does not only play a key role in the cycling and transforming process of nutrients but also serves as the most important ''warehouse'' and ''source'' of nutrient elements suggesting the effective status of soil nutrients and the change of biological activity after the soil is affected by the external world (Jenkinson and Ladd, 1981). Soil physico- chemical characteristics influence the composition of the soil microbial community, their activity and the level of microbial biomass (Schnurer *et al*., 1985; Dick,1994). It is important to determine optimum diversities of soil microbial populations of forest systems for their sustainable management. In order to maximize the beneficial effects of microbial activity, there is a need for greater understanding of factors influencing microbial communities and their activities. The relationship between soil microbial communities particularly fungi and bacteria and their activities, plant quality and ecosystem sustainability of broad leaved forests are still poorly understood in forest stands of Meghalaya, India. The present study was undertaken

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to obtain a better understanding of the interactions between fungi and bacteria and microbial biomass carbon and nitrogen, microbial respiration, various environmental factors in the forests soil of Meghalaya, India.

MATERIALS AND METHODS

Study sites: Two broad leaved forest stands differing in altitudes were selected for the present investigation. They were (i) Upper Shillong (1861 msl altitude) and (ii) Mawkyrdep (altitude 889 msl).

Soil samplings: The soil samples were collected from the two study sites at two different depths i.e., 0 - 10 cm and 10 - 20 cm for a period of two years i.e., 2009 and 2010 and the following studies were carried out:

Isolation, identification and estimation of microbial populations (fungi and bacteria) from the soil

For the isolation of fungi and bacteria, soil plate method Warcup (1950) using Rose Bengal Agar Medium Martin (1950) and serial dilution plate method Johnson and Curl (1972) using Nutrient Agar medium were followed respectively. Colony forming units (CFUs) of fungi and bacteria were calculated on dry weight basis using the following formulae:

$$\text{CFU of fungi g}^{-1}\text{ dry weight} = \frac{\text{Number of colonies}}{\text{Dry weight of soil (g)}}$$

$$\text{CFU of bacteria g}^{-1}\text{ dry weight} = \frac{\text{Number of colonies x dilution factor x inoculum}}{\text{Dry weight of soil (g)}}$$

Soil microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) were determined using the chloroform-fumigation method given by Anderson and Ingram (1993). Soil respiration was determined by the absorption and titration method Macfayden (1970). The following indices for fungi and bacteria species structure were also calculated:

(a) Index of general diversity (H') or Shannon and Weaver (1948) diversity index

$$H' = \Sigma\ (ni/\ N \log ni\ N)$$

(Where *ni* is the importance value of each species and *N* is the total importance value)

(b) Index of dominance (C) or Simpson (1949) index of dominance.

$$C = \Sigma\ (ni/\ N)\ 2$$

(Where *ni* is the importance value of each species and *N* is the total importance value)

Soil Physico- Chemical Properties

Soil temperature was noted using soil thermometer at the time of sampling. The moisture content was determined by drying the samples in hot air oven at 105°C for 24h respectively. Soil pH was read using electronic digital pH meter. Organic carbon was measured by the method given by Anderson and Ingram (1993), Total nitrogen (N), available phosphorus (P) and exchangeable potassium (K) by the methods of micro- kjeldahl distillation Anderson and Ingram (1993) molybdenum blue Allen and K by flame photometer Jackson (1972) respectively. Statistical analyses of the data were performed using Statistica 8.0 software package.

RESULTS

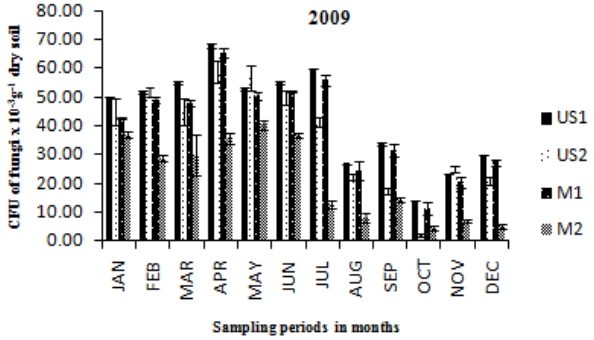
Colony forming units (CFU) of fungi and bacteria of the soil

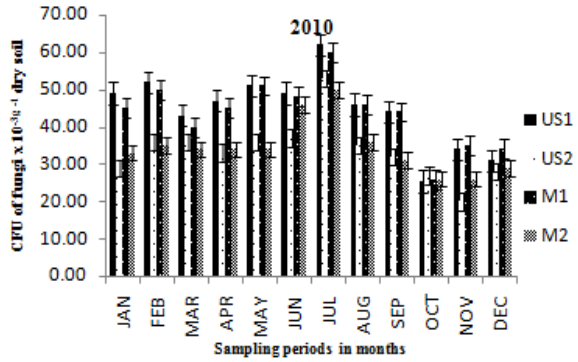
Both fungal and bacterial CFUs exhibited monthly variations in the soils of the two different forest stands during the study periods of 2009 and 2010. The soil at the high altitude forest stand harbored higher fungal and bacterial CFUs as compared to that at low altitude forest stand. In the first year of the study period, at 0- 10 cm depth at both the forest stands and 10- 20 cm depth at the high altitude forest stand, the maximum fungal CFU was observed in the month of April and the minimum was observed in the month of October, whereas, at the low altitude forest stand, at 10- 20 cm depth, the maximum fungal CFU was observed in the month of May and the minimum was observed in the month of October. In the second year of the study period, at 0- 10 cm and 10- 20 cm depth at both the forest stands, maximum fungal CFU was observed in the month of July and the minimum was observed in the month of October and November at 0- 10 cm and 10- 20 cm depth respectively at both the forest stands. The fungal CFU decreased with increase in depth (Fig. 1).

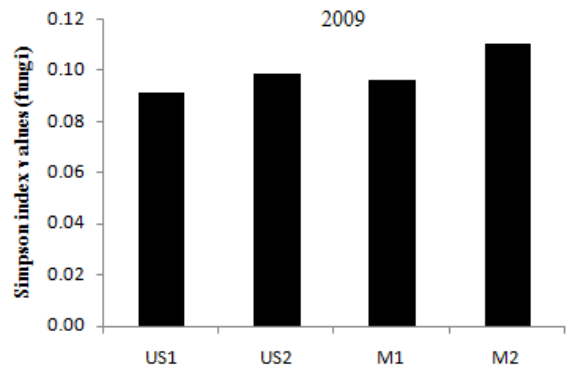
In the first year of the study period, at 0- 10 cm depth, the maximum bacterial CFU was observed in the month of April and the minimum was observed in the month of December. At 10- 20 cm depth, the maximum bacterial CFU was observed in the month of May and the minimum was observed in the month of October at the high altitude forest stand, whereas, at the low altitude forest stand at 0- 10 cm depth, the maximum bacterial CFU was observed in the month of April and the minimum was observed in the month of October. At 10- 20 cm depth, the maximum bacterial CFU was observed in the month of May and the minimum was observed in the month of October. In the second year of the study period, at 0- 10 cm and 10- 20 cm depth, the maximum bacterial CFU was observed in the month of July at both the forest stands and the minimum was observed in the month of October at the high altitude forest stand, whereas, at the low altitude forest stand, at 0- 10 cm and 10- 20 cm depth, the minimum was observed in the month of November. The bacterial CFU also decreased with increase in depth (Fig. 4). Table 1 depicts list of fungal species isolated from the soils of both the forest stands at two different depths i.e. 0- 10 cm and 10- 20 cm. Altogether, 110 fungal species were isolated from the two different depths (0- 10 cm and 10- 20 cm) at the two forest stands.

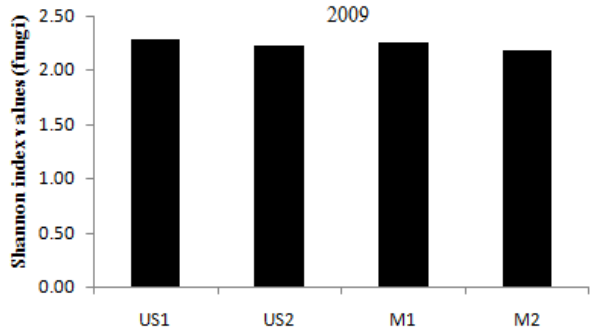
Maximum fungal genera isolated belonged to Deuteromycotina (15 genera, 68 species) followed by Ascomycotina (13 genera, 17 species) and Zygomycotina (5 genera, 17 species), Mastigomycotina (1 genus, 8 species). Highest number of species of *Penicillium* (25 species), could be isolated followed by *Aspergillus* (9 species), *Pythium* (8 species), *Fusarium* (7 species), *Mucor* (6 species), *Mortierella* and *Trichoderma* (5 species each), *Absidia, Acremonium, Cladosporium, Eupenicillium, Gliocladium, Phoma* (3 species each), *Humicola, Nectria, Oideodendron, Paecilomyces, Rhizopus* and *Talaromyces* (2 species each) and I species each of *Alternaria, Chaetomium, Emericella, Gongronella, Gonytrichum, Leptospherella, Monographella, Pestilotia, Phialophora, Pseudoeurotium, Ramichloridium, Scopulariopsis, Staphylotrichum, Torula* and *Verticillium*. Majority of the fungal species isolated were common to both the forest stands. The dominant fungal species isolated at 0- 10 cm depth of soil at the high altitude forest stand were *Acremonium cerealis, Fusarium poae, Humicola grisea, Mucor circinelloides, Penicillium frequentans, P. janthinellum, P. lanosum, Pythium aphanidermatum* and *Trichoderma viride.* At 10- 20 cm depth, the dominant fungal species isolated were *Acremonium cerealis, Humicola fuscoatra, Penicillium canescens, P. janthinellum, P. lanosum* and *P. verrucossum.* At 0- 10 cm depth of soil at the low altitude forest stand, the dominant fungal species isolated were *Acremonium cerealis, Aspergillus flavus, A. fumigatus, Cladosporium herbarum, Gliocladium roseum, Mucor circinelloides, Penicillium frequentans, Pythium aphanidermatum, Trichoderma koningii* and *T. viride.* At 10- 20 cm depth of soil, the dominant fungal species

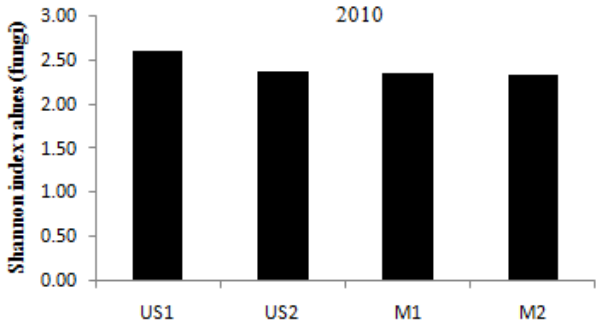
isolated were *Acremonium cerealis, Aspergillus flavus, A. fumigatus, Humicola fuscoatra, Mucor circinelloides, Penicillium janthinellum, P. lanosum, Trichoderma koningii* and *T. viride* Qualitatively, there was not much difference in the composition of fungal flora. However, few fungal species were restricted to each study site. *Absidia glauca, Aspergillus candida, Emericella nidulans Eupenicillium javanicum, E. lapidosum, Fusarium chlamydosporum, F. semitectum, F. solani, Mortierella exigua, M. polycephala, Mucor polycephala, M. racemosus, Phoma exigua, Phialophora cyclaminis, Ttrichosporiella* and *Verticillium albo atrum* were restricted at 0- 10 cm depth of soil at the high altitude forest stand. *Aspergillus alutaceus, Penicillium waksmanii, Pythium cinamomii, Rhizopus oryzae* and *Trichoderma harzianum* were restricted at 10- 20 cm depth of soil at the high altitude forest stand. At the low altitude forest stand, restricted fungal species include *Aspergillus restrictus, Chaetomium tetrasporum, Fusarium culmorum, F. trinctum, Gonytrichum, Paecilomyces carneus, P. lilacinus, Penicillium corylophilum, P. sacculum, Pseudoeurotium zonatum, Pythium ultimum, Ramichloridium schulzenii, Mortierella ramanniana, Talaromyces emersonii* and *Trichoderma polyspora* at 0- 10 cm depth of soil. *Mortierella minutissima, Nectria inventa, Penicillium claviforme, P. expansum, P. implicatum, P. rubrum P. variabile, Pestilotia, Phoma pomorum* and *Pythium inflatum* were restricted at 10- 20 cm depth of soil.

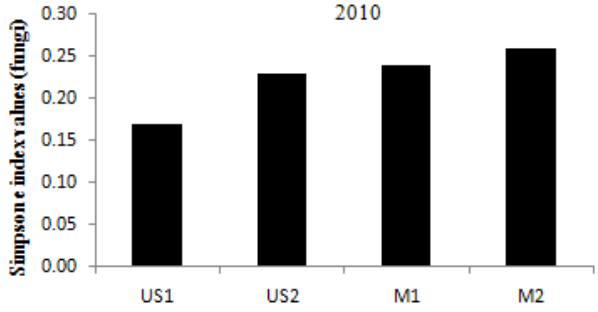


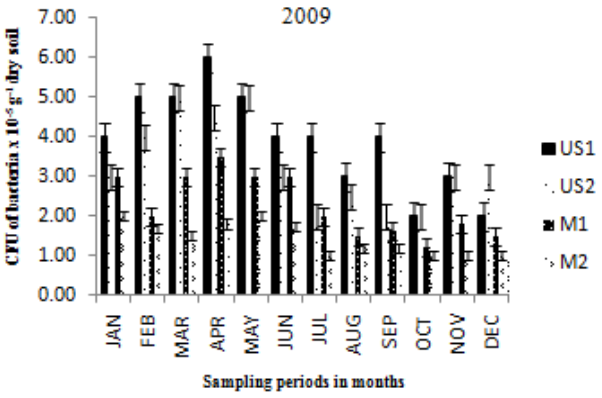












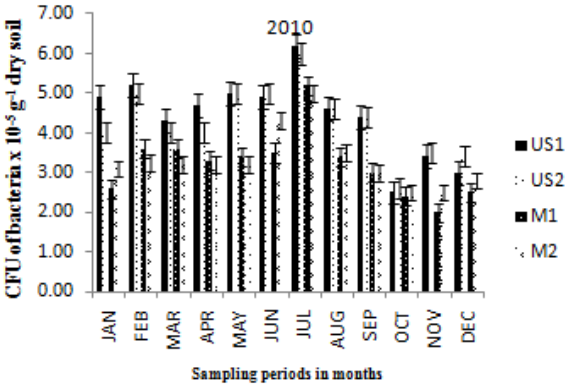


Table 1. List of fungal species isolated at two different depths (0-10cm and 10-20cm) of soil at the two forest stands during the study periods of 2009 and 2010

Sl. No.	Fungal species	2009				2010			
		US1	US2	M1	M2	US1	US2	M1	M2
	Mastigomycotina (1 genus, 8 species)								
1	*Pythium aphanidermatum*	+	+	+	+	+	+	+	+
2	*P. carolinianum*	-	-	-	-	+	-	-	-
3	*P. cinnamomii*	-	-	-	-	-	+	-	-
4	*P. inflatum*	-	-	-	-	-	-	-	+
5	*P. intermedium*	-	-	-	+	+	+	+	+
6	*P. irregulare*	-	-	-	-	+	+	+	-
7	*P. paroecandrum*	-	-	-	-	-	+	-	-
8	*P. ultimum*	-	-	-	-	-	-	+	-
	Zygomycotina (5 genera, 17 species)								
1	*Absidia corymbifera*	+	+	-	+	+	-	-	+
2	*A. cylindrospora*	+	-	+	+	+	-	-	-
3	*A. glauca*	+	-	-	-	-	-	-	-
4	*Gongronella butleri*	-	-	-	-	+	+	-	-
5	*Mortierella exigua*	-	-	-	-	+	+	-	-
6	*M. gamsii*	-	-	-	-	+	+	-	-
7	*M. minutissima*	-	-	-	-	-	-	-	+
8	*M. polycephala*	-	-	-	-	+	-	-	-
9	*M. ramanniana*	+	-	-	-	-	-	-	-
10	*Mucor circinelloides*	+	+	+	+	+	+	+	-
11	*M. hiemalis*	+	+	-	-	-	-	+	-
12	*M. mucedo*	+	+	-	-	-	-	-	-
13	*M. polyspora*	-	-	-	-	+	-	-	-
14	*M. racemosus*	-	-	-	-	+	-	-	-
15	*M. vinacea*	-	-	-	-	-	+	-	-
16	*Rhizopus oryzae*	-	+	-	-	-	-	-	-
17	*R. stolonifer*	+	-	+	-	-	-	-	-
	Ascomycotina (13 genera, 17 species)								
1	*Chaetomium tetrasporum*	-	-	-	-	-	-	+	-
2	*Emericella nidulans*	+	-	-	-	-	-	-	-
3	*Eupenicillium brefeldiadum*	+	-	-	-	-	+	-	-
4	*E. javanicum*	-	-	-	-	+	-	-	-
5	*E. lapidosum*	-	-	-	-	+	-	-	-
6	*Leptosphaeria maculans*	-	-	-	-	-	-	+	+
7	*Monographella nivalis*	-	-	-	-	-	-	+	-
8	*Nectria inventa*	-	-	-	-	-	-	-	+
9	*N. ventricosa*	-	-	+	-	-	-	+	
10	*Pestilotia* sp.	-	-	-	-	-	-	-	+
11	*Phialophora cyclaminis*	-	-	-	-	+	-	-	-
12	*Pseudoeurotium zonatum*	-	-	-	-	-	-	+	-
13	*Ramichloridium schulzenii*	-	-	-	-	-	-	+	-
14	*Scopulariopsis brumptii*	+	-	-	+	-	-	-	-
15	*Talaromyces emersonii*	-	-	-	-	-	-	+	-
16	*T. trachyspermus*	-	-	-	+	-	-	-	-
17	*Torula herbarum*	-	-	-	-	-	-	+	-
	Deuteromycotina (15 genera, 68 species)								
1	*Aceremonium butyri*	+	-	+	-	-	+		-
2	*A. cerealis*	+	+	+	-	+	+	+	+
3	*A. murorum*	-	-	-		+	+	+	-
4	*Alternaria alternata*	+	-	+	+	-	+	-	-
5	*Aspergillus alutaceus*	-	-	-	-	-	+	-	-
6	*A. candida*	-	-	-	-	+	-	-	-
7	*A. flavus*	+	+	+	+	-	-	-	-
8	*A. fumigatus*	+	+	+	+	-	+	-	-
9	*A. niger*	+	+	+	+	-	+	-	-
10	*A. restrictus*	-	-	-	-	-	-	+	
11	*A. sydowii*	-	+	-	-	-	+	-	+
12	*A. versicolor*	-	+	-	-	-	-	-	-
13	*A. wentii*	-	-	+	-	+	-	-	-
14	*Cladosporium cladosporioides*	+	+	-	+	-	+	+	+
15	*C. herbarum*	+	+	+	+	+	+	+	+
16	*C. macrocarpum*	-	-	+	+	+	-	-	-
17	*Fusarium chlamydosporum*	-	-	-	-	+	-	-	-
18	*F. culmorum*	-	-	-	-	-	-	+	-
19	*F. oxysporum*	-	-	-	-	+	+	+	+
20	*F. poae*	-	-	-	-	-	-	+	+
21	*F. semitectum*	-	-	-	-	+	-	-	-
22	*F. solani*	-	-	-	-	+	-	-	-
23	*F. trinctum*	-	-	-	-	-	-	+	-
24	*Gliocladium catenulatum*	-	-	+	-	+	-	+	-
25	*G. roseum*	-	-	-	-	-	+	+	
26	*G. viride*	-	-	-	-	-	+	-	+
27	*Gonytrichum`*	-	-	+	-	-	-	-	-
28	*Humicola fuscoatra*	+	+	+	+	+	+	+	+
29	*H. grisea*	+	+	+	+	+	+	+	+
30	*Oideodendron echinulatum*	-	-	+	+	-	-	-	-

31	*O. tennuissimum*	-	-	+	+	-	-	-	-
32	*Paecilomyces carneus*	-	-	+	-	-	-	+	-
33	*P. lilacinus*	-	-	-	-	+	-	+	-
34	*Penicillium atrovenetum*	+	+	-	-	+	+	+	+
35	*P. brevicompactum*	+	+	+	+	-	-	+	-
36	*P. canescens*	+	+	+	+	+	+	+	-
37	*P. chrysogenum*	-	+	+	-	+		-	-
38	*P. citrinum*	-	-	-	-	+	+	-	-
39	*P. claviforme*	-	-	-	-	-	-	-	+
40	*P. corylophilum*	-	-	-	-	-	-	+	+
41	*P. dalae*	-	-	-	-	-	+	-	+
42	*P. expansum*	-	-	-	-	-	-	-	+
43	*P. fellutanum*	+	+	-	+	-	-	-	-
44	*P. frequentans*	+	+	+	+	+	+	+	+
45	*P. funiculosum*	-	-	-	-	-	+	-	+
46	*P. granulatum*	-	-	-	-	-	+	-	+
47	*P. implicatum*	-	-	-	-	-	-	-	+
48	*P. janthinellum*	+	+	+	+	+	+	-	+
49	*P. lanosum*	+	+	+	+	+	+	+	-
50	*P. purpurogenum*	+	+	-	+	-	+	-	+
51	*P. restrictum*	-	-	-	-	-	+	-	-
52	*P.rubrum*	-	-	-	-	-	-	-	+
53	*P. sacculum*	-	-	+	-	-	-	-	-
54	*P. simplissisimum*	+	+	-	+	-	-	+	-
55	*P. stoloniferum*	+	+	+	-	+	-	+	-
56	*P. variabile*	-	-	-	-	-	-	-	+
57	*P. verrucossum*	+	+	+	+	+	+	+	+
58	*P. waksmanii*	-	-	-	-	-	+	-	-
59	*Phoma eupyrena*	+	-	-	-	-	-	+	+
60	*P. exigua*	-	-	-	-	+	-	-	-
61	*P. pomorum*	-	-	-	-	-	-	-	+
62	*Staphylotrichum coccosporum*	-	-	+	-	-	-	+	-
63	*Trichoderma hamatum*	-	-	-	-	-	+	+	+
64	*T. harzianum*	-	-	-	-	-	+	-	-
65	*T. koningii*	+	+	+	+	+	+	+	+
66	*T. polyspora*	-	-	-	-	-	-	+	+
67	*T. viride*	+	+	+	+	+	+	+	-
68	*Verticillium albo atrum*	-	-	-	-	+	-	-	-

Note: '+' indicates present
'-' indicates absent
US1= Upper Shillong (high altitude) 0- 10 cm depth of soil; US2= Upper Shillong (high altitude) 10- 20 cm depth of soil
M1= Mawkyrdep (low altitude) 0- 10 cm depth of soil; M2= Mawkyrdep (low altitude) 10- 20 cm depth of soil

Table 2. Values (range) of biochemical properties at two different depths (0-10cm and 10-20cm) of soil at the two forest stands During the study periods of 2009 and 2010. Values in the parentheses indicate the mean and standard error.

Soil biological and biochemical properties	Year	US1	US2	M1	M2
SR (mg/kg^{-1} soil $24h^{-1}$)	2009	63.80-76.00 (72.76±0.84)	61.97-75.17 (70.49±0.59)	59.40-75.00969 (69.03±0.84)	58.67-71.87 (67.76±0.84)
	2010	68.93-76.27 (73.35±0.93)	66.00-74.80 (69.98±1.08)	64.90-72.60 (68.81±0.97)	62.33-73.33 (67.08±0.90)
C_{mic} ($\mu g\ Cg^{-1}$)	2009	235.00- 750.00 (502.92±1.78)	200.00-590.00 (414.17±0.07)	200.00-690.00 (451.67±0.74)	140.00-500.00 (314.75±0.13)
	2010	300.00-600.00 (450.00±0.29)	250.00-500.00 (364.17±0.84	180.00-550.00 (368.33±0.11)	170.00-400.00 (268.75±0.00)
N_{mic} ($\mu g\ Ng^{-1}$)	2009	32.00-85.00 (62.17±0.13)	31.00-99.00 (64.67±0.22)	25.00-65.00 (48.92±0.22)	30.00-68.00 (51.42±0.16)
	2010	30.00-58.00 (44.33±0.21)	26.00-68.00 (43.17±0.15)	35.00-62.00 (47.50±0.15)	25.00-58.00 (41.00±0.19)

Table 3. Values (range) of physico-chemical properties at two different depths (0-10cm and 10-20cm) of soil at the two forest stands during the study periods of 2009 and 2010. Values in the parentheses indicate the mean and standard error

Soil properties	Year	US1	US2	M1	M2
Soil temperature (°C)	2009	8.00- 18.00 (12.75± 0.12)	8.00- 17.50 (11.88± 0.85)	12.00-24.00(16.92± 0.12)	12.00-23.00(16.58±0.87)
	2010	7.00-17.30(12.26±0.02)	7.00-17.00(10.98±0.01)	13.00-24.00(18.13±0.01)	11.00-21.90(16.39±0.02)
Moisture content (%)	2009	23.80-50.00(41.00±0.09)	21.00-51.47(42.37±0.08)	23.26-50.00(41.00±0.09)	18.67-40.67(25.88±0.10)
	2010	16.24-50.47(34.15±0.15)	15.47-49.43(34.08±0.12)	10.56-43.83(24.13±0.12)	16.14-47.03(24.54±0.07)
pH	2009	4.83-5.67(5.30±0.10)	4.86-5.57(5.30±0.20)	5.23-6.07(5.56±0.20)	4.90-5.73(5.40±0.20)
	2010	4.67-5.62(5.00±0.08)	5.70-4.57(5.08±0.10)	5.58-6.26(4.59±0.10)	4.98-6.00(5.40±0.09)
Organic carbon (%)	2009	2.03-5.64(3.93±0.18)	1.97- 4.28(3.39±0.16)	1.39-3.47(2.34±0.20)	1.39- 3.11(2.08±0.20)
	2010	2.40- 3.51(4.72±0.98)	2.10- 4.21(3.42±0.78)	2.00-3.70(3.18±0.17)	4.00-2.60(3.28±0.12)
Available phosphorus (%)	2009	0.044- 0.124(0.086±0.10)	0.036- 0.122(0.075±0.03)	0.024-0.124(0.080±0.19)	0.056- 0.108(0.082±0.10)
	2010	0.00046- 0.00096 (0.0007167±0.09)	0.00055-0.0014 (0.00096±0.06)	0.000440- 0.00140 (0.000920±0.12)	0.00056- 00104 (0.000727±0.12)
Total nitrogen (%)	2009	0.011- 0.026 (0.020±0.100	0.010-0.021 (0.014±0.09)	0.008- 0.012 0.017±0.012)	0.006- 0.0222 (0.0152±0.0)
	2010	0.015- 0.035 (0.0220±0.13)	0.011- 0.025 (0.018±0.12)	0.005- 0.025 (0.0172±0.100)	0.005- 0.030 (0.014±0.11)
Exchangeable potassium (%)	2009	0.06-1.32 (0.862±0.09)	0.33- 0.83 (0.52±0.10)	0.65- 2.13 (1.378±0.10)	0.46- 1.38 (0.73±0.15)
	2010	0.19-0.37 (0.293±0.11)	0.11- 0.37 (0.177±0.08)	0.33- 1.19 (0.816±0.10)	0.36- 0.87 (0.589±0.09)

Table 4. Correlation coefficient (r) values of fungal and bacterial CFUs with the various and biochemical and physico-chemical properties of soil at two different depths (0-10cm and 10-20cm) at the two forest stands during the study periods of 2009 and 2010

Study sites	Microbial population	Year	BP	C_{mic}	N_{mic}	SR	ST	MC	PH	OC	TN	AP	K
US1	FP	2009	0.86^{b}	0.96^{c}	0.67^{a}	-	-	0.72^{a}	-	0.77^{a}	-	0.75^{a}	-
		2010	0.99^{c}	0.71^{a}	0.80^{b}	-	-	0.80^{b}	-	0.70^{a}	-	-	-
	BP	2009	-	0.81^{b}	0.70^{a}	-	-	0.70^{a}	-	0.77^{a}	-	0.58^{a}	-
		2010	-	0.71^{a}	0.80^{b}	-	-	0.80^{b}	-	0.70^{a}	0.58^{a}	-	-
US 2	FP	2009	0.72^{a}	0.76^{a}	-	-	-	0.58^{a}	-	0.70^{a}	-	-	-
		2010	0.97^{c}	0.80^{b}	0.80^{b}	-	-	-	-	0.64^{a}	0.62^{a}	-	-
	BP	2009	-	-	-	-	-	-	-	-	-	-	-
		2010	-	0.88^{b}	0.73^{a}	-	-	-	-	0.59^{a}	0.56^{a}	-	-
M 1	FP	2009	0.81^{b}	0.92^{c}	0.59^{a}	0.65^{a}	-	0.70^{a}	-	-	0.77^{a}	-	-
		2010	0.98^{c}	0.82^{b}	0.61^{a}	-	-	-	-	0.62^{a}	0.66^{a}	-	0.65^{a}
	BP	2009	-	0.87^{b}	-	-	-	-	-	-	0.73^{a}	0.59^{a}	-
		2010	-	0.82^{c}	0.68^{a}	-	-	-	-	0.63^{a}	0.64^{a}	-	-
M 2	FP	2009	0.96^{c}	0.99^{c}	-	-	-0.57^{a}	-	-	0.60^{a}	0.81^{b}	-	0.81^{b}
		2010	0.99^{c}	0.94^{c}	0.62^{a}	-	-0.54^{a}	-	-	0.88^{c}	0.79^{b}	-	-
	BP	2009	-	-	0.96^{c}	-	-	-	-	0.66^{a}	0.70^{a}	-	0.70^{a}
		2010	-	0.95^{c}	0.60^{a}	-	-	-	-	0.89^{b}	0.75^{a}	-	-

Insignificant values are marked with '-'

Values marked with a, b and c indicate significant correlations at $p<0.05$, 0.01 and 0.001 respectively

Note: FP= Fungal population; BP= Bacterial population;US1= Upper Shillong (high altitude) 0- 10 cm depth of soil; US2= Upper Shillong (high altitude) 10- 20 cm depth of soil; M1=Mawkyrdep (low altitude) 0- 10 cm depth of soil; M2= Mawkyrdep (low altitude) 10- 20 cm depth of soil; C_{mic}= Soil microbial biomass carbon, N_{mic} = Soil microbial biomass nitrogen; SR= Soil respiration; MC= moisture content; OC= organic carbon; TN= total nitrogen; AP= available phosphorus; K= exchangeable potassium.

Table 5. One way analysis of variance (ANOVA at $p \leq 0.05$) of soil microbial populations, soil microbial biomass (carbon and nitrogen), soil respiration and physico-chemical properties at two different depths (0-10cm and 10-20cm) of soil at the two forest stands during the study periods of 2009 and 2010

Microbial populations	Year	Source of variation	F- value	P- level
	2009	US1 x US2 x M1 x M2	3.9955	0.013357
	2010		4.8588	0.005262
Fungi	2009	US1 X M1	-	-
	2010		8.7815	0.007178
	2009	US2 x M2	4.84821	0.038457
	2010		5.6885	0.026121
	2009	US1 x US2 x M1 x M2	16.1000	0.000001
	2010		6.4763	0.000999
Bacteria	2009	US1 X M1	15.3131	0.000745
	2010		10.3321	0.003995
	2009	US2 x M2	28.1216	0.000025
	2010		-	0.006828
Biochemical properties				
C_{mic}	2009	US1 x US2 x M1 x M2	-	-
	2010		7.8790	0.000258
	2009	US1 X M1	5.028	0.035345
	2010		-	-
	2009	US2 x M2	-	-
	2010		10.7688	0.003408
N_{mic}	2009	US1 x US2 x M1 x M2	3.4600	0.024169
	2010		-	-
	2009	US1 X M1	5.7382	0.025544
	2010		-	-
	2009	US2 x M2	4.4484	0.046545
	2010		-	-
Soil respiration	2009	US1 x US2 x M1 x M2	3.61	0.020545
	2010		13.90	0.000002
	2009	US1 X M1	5.028	0.035345
	2010		26.29	0.000039
	2009	US2 x M2	-	-
	2010		6.81	0.016004
		Physico- chemical properties of soil		
Soil Temperature (° C)	2009	US1 x US2 x M1 x M2	7.5196	0.000362
	2010		11.9500	0.000007
	2009	US1 X M1	9.2717	0.005941
	2010		17.7863	0.000355
	2009	US2 x M2	13.0647	0.001537
	2010		15.6310	0.000675
	2009	US1 x US2 x M1 x M2	8.9592	0.000095
	2010		5.0907	0.004119

Moisture content (%)	2009	US1 X M1	-	-
	2010		8.4364	0.008219
	2009	US2 x M2	-	-
	2010		6.9082	0.015349
PH	2009	US1 x US2 x M1 x M2	-	-
	2010		7.97	0.000236
	2009	US1 X M1	4.733	0.040616
	2010		15.461	0.000712
	2009	US2 x M2	-	-
	2010		6.562	0.017792
Organic carbon (%)	2009	US1 x US2 x M1 x M2	13.4178	0.000002
	2010		-	-
	2009	US1 X M1	18.0399	0.000330
	2010		-	-
	2009	US2 x M2	19.8186	0.000200
	2010		-	-
Total nitrogen (%)	2009	US1 x US2 x M1 x M2	3.8585	0.015528
	2010		3.3306	0.027941
	2009	US1 X M1	-	-
	2010		-	-
	2009	US2 x M2	-	-
	2010		-	-
Available phosphorus (%)	2009	US1 x US2 x M1 x M2	-	-
	2010		3.5801	0.021140
	2009	US1 X M1	-	-
	2010		-	-
	2009	US2 x M2	-	-
	2010		6.3949	0.019122
Exchangeable potassium (%)	2009	US1 x US2 x M1 x M2	78.4854	0.00
	2010		48.8253	0.000000
	2009	US1 X M1	11.3050	0.002813
	2010		55.3048	0.000000
	2009	US2 x M2	-	-
	2010		89.0514	0.000000

Insignificant values are marked with '-'
Note: US1= Upper Shillong (high altitude) 0- 10 cm depth of soil; US2= Upper Shillong (high altitude) 10- 20 cm depth of soil; M1= Mawkyrdep (low altitude) 0- 10 cm depth of soil; M2= Mawkyrdep (low altitude) 10- 20 cm depth of soil

Statistical analysis

Table 4 depicts the overall statistical analyses amongst various parameters studied. In the first year of study period at the high altitude forest stand, CFU of fungi and bacteria at 0- 10 cm depth showed positive significant correlations with moisture content, organic carbon, available phosphorus, soil microbial biomass carbon and nitrogen. At 10-20 cm depth, CFU of fungi showed positive significant correlations with moisture content, organic carbon and soil microbial biomass carbon. At the low altitude forest stand, at 0-10 cm depth, CFU of fungi showed positive significant correlations with moisture content, soil respiration, total nitrogen, soil microbial biomass carbon and nitrogen. CFU of bacteria showed positive significant correlations with moisture content, total nitrogen, soil respiration, soil microbial biomass carbon and nitrogen. At 10- 20 cm depth, CFU of fungi showed positive significant correlations with soil temperature, organic carbon, total nitrogen, exchangeable potassium and soil microbial biomass carbon. CFU of bacteria showed positive significant correlations with organic carbon, total nitrogen, exchangeable potassium and microbial biomass carbon. In the second year of the study period at high altitude forest stand, CFU of fungi at 0- 10 cm depth showed positive significant correlations with moisture content, organic carbon, soil microbial biomass carbon and nitrogen. CFU of bacteria showed positive significant correlations with moisture content, organic carbon, total nitrogen, soil microbial biomass carbon and nitrogen.

At 10- 20 cm depth, CFU of fungi and bacteria showed positive significant correlations with organic carbon, total nitrogen, soil microbial biomass carbon and nitrogen. At the low altitude forest stand, at 0- 10 cm and 10- 20 cm depth, CFU of fungi showed positive significant correlations with organic carbon, total nitrogen, exchangeable potassium, soil microbial biomass carbon and nitrogen. At 10- 20 cm depth, CFU of fungi showed positive significant correlations with soil temperature, organic carbon, total nitrogen, soil microbial biomass carbon and nitrogen. CFU of bacteria at 0- 10 cm and 10- 20 cm depth showed positive significant correlations with organic carbon, total nitrogen, soil microbial biomass carbon and nitrogen. Shannon diversity index showed that the highest fungal diversity was observed at the surface layer of both the forest stands. The trend showed decreasing order as US1> M1> US2 > M2 in 2009 and US1> US2 > M1> M2 in 2010. Simpson dominance index was found to be highest in M2, followed by US2, M1 and US1 in 2009 and it was highest in M2, followed by M1, US2 and US1 in 2010. (Fig. 2 and 3). The one way analysis of variance (ANOVA) showed significant variation ($p \leq 0.05$) of fungal and bacterial CFUs at all the soil depths (US1 x US2 x M1 x M2, US1 x M1 and US2 x M2) of both the forest stands during the study periods 2009 and 2010 (Table 5).

Biochemical properties of soil (Microbial biomass carbon and nitrogen and soil respiration)

Both microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) exhibited monthly variations at the two different forest stands during the study periods. The soil at the high altitude forest stand exhibited higher C_{mic} and N_{mic} as compared to that at the low altitude forest stand and decreased with the increase in depth. There was not much variation in the soil respiration at both the study sites throughout the study periods. Soil respiration was observed to be higher at the high altitude forest stand as compared to that at the low altitude forest stand. Soil respiration also decreased with increase in depth (Table 2). Microbial biomass carbon and nitrogen and soil respiration showed positive significant correlations with CFUs of fungi and bacteria and physico- chemical properties (Table 4). One way analysis of variance (ANOVA) showed significant variations ($p \leq 0.05$) of microbial

biomass carbon and nitrogen and soil respiration between the soils at the two different depths at both the forest stands (Table 5).

Physico- chemical properties of soil (temperature, pH, moisture content, organic carbon, available phosphorus, total nitrogen and exchangeable potassium)

It was observed that the soil at the low altitude forest stand exhibited higher temperature as compared to that at the high altitude forest stand. Higher soil temperature was recorded at the surface soil as compared to the sub- surface layer. pH was more acidic at the high altitude forest stand. Except exchangeable potassium, moisture content, organic carbon, available phosphorus and total nitrogen were higher at the high altitude forest stand as compared to that at the low altitude forest stand (Table 3). Physico- chemical properties showed positive correlations with biochemical properties and CFUs of fungi and bacteria (Table 4). One way analysis of variance (ANOVA) showed significant variations ($p \leq 0.05$) of physico- chemical properties between the soils at the two different depths at both the forest stands (Table 5).

DISCUSSIONS

The higher fungal and bacterial CFUs observed at the high altitude forest stand could be due to better availability of nutrients and environmental conditions which favored their growth. Maximum fungal and bacterial CFUs in the months of April and July at both the study sites during the study period could be due to the climatic conditions and availability of substrates during these months which appear to favor the growth and development of the soil microbes (Wright, 1961). Tiwari (1991) also reported highest number of microbes in spring- summer season while least number of fungi was recorded during winter season. The soil moisture content was related to the fungal CFU and was responsible for the higher microbial population number during the rainy season. Selvam (2010) also reported moisture content had positive correlations with microbial populations and other soil properties. The higher diversity of fungi in the surface soil depth indicated by the Shannon diversity index can be ascribed to the relatively large amount of nutrients (organic matter and possibly limiting nutrients) in the surface depth than in the sub-surface soil depth enhancing the microbial ability to withstand environmental perturbations. The increase in soil temperature at the low altitude forest stand resulted in decreased in fungal and bacterial CFUs. The lower number of fungal and bacterial CFUs during winter months could be due to the low soil moisture in the dry winter season.

Soil microbial population was less during periods when temperature and moisture conditions are low, while it peaked during rainy season when the litter decomposition rate is at its peak on the forest floor. The seasonal variation in the fungal spectrum of the soil might be due to seasonal variations in soil moisture, temperature, pH and organic matter of the soil. Liu (2000) stated soil moisture, soil temperature and substrate availability as the most important factors that influence soil microbial growth and population density. Lower fungal and bacterial CFUs during winter at both the study sites may be ascribed to low moisture content during these periods. It was observed that the surface layer had higher fungal and bacterial CFUs as compared to that at the sub- surface layer at both the forest stands. The higher fungal and bacterial CFUs at the surface layer might be due to the presence of litters, twigs, herbs and tree canopy which render a moist environment in the soil and favor high microbial activity and hence high microbial populations. Fungal and bacterial CFUs tend to decrease with increase in soil depth. Decrease in the fungal and bacterial CFUs with increasing soil depth could be related to the organic carbon content of the soil as nutrients are declining with the increase in soil depth. Among several factors affecting microbial population and activity, moisture, temperature, nutrient regime and soil depth are important factors. Microbial populations generally decrease downwards through the soil profile, which is a trend that has also been reported in forest soil profiles (Richter and Markewitz, 1995; Ekelund *et al*., 2001) indicating less favorable conditions for microbial activities at lower soil depths. Bell (2008) suggested that variability in fungal activity was related to soil temperatures ranging between 13° and 26 °C. Selvam (2010) also reported that it could possibly be due to the decreasing of aeration and substrate supply with the increasing of soil depth. These findings indicate that changes in soil moisture, coupled with soil temperatures and resource availability, drive the functioning of soil-microbial dynamics. Previous studies by Schimel (1999) have shown that the variability in soil moisture content can influence the composition of soil bacteria and fungal communities. The high microbial biomass carbon and nitrogen at the high altitude forest stand as compared to that at the low altitude forest stand could be due to the surface run off of the adjacent hill areas. The microbial biomass carbon was found to be higher in the surface soil layer and decreased with increase in depth. Maithani (1996) also observed that the surface soil layer (0-10 cm) had significantly higher microbial biomass carbon than the sub-surface layer. Large pool of organic matter at the soil surface supports a uniquely large and active soil microbial community.

Higher soil respiration at the high altitude forest stand as compared to that at the low altitude forest stand reflect the favourable effect of soil moisture. Dkhar and Mishra (1987) reported a significant correlation between carbon dioxide evolution and microbial population. Dullinger *et al*. (2007) have also indicated that increased soil respiration results in higher soil microbial community composition. Increased soil temperature at the two study sites during the summer months could be due to effect of the solar radiation and the heating up of the surrounding soil surface. Studies have shown that nearer the angle of incidence of the sun's rays approaches the perpendicular, the greater will be the absorption. Variation in the soil temperature was recorded at both the forest stands; however, it was observed that the surface soil layers showed more temperature than the sub- surface layers. A thick layer of ground vegetation limit amount of heat the soil can absorb during the summer. Other soil characteristics, such as soil moisture, may also be involved. Soil temperatures can provide a long term measure of how the climate is changing because soil temperatures, especially at deeper levels tend to reflect long term changes. Similar trend of temporal and depth wise variations in soil temperature and soil pH was noted by Baruah and Dkhar (1983) in rice and maize fields respectively. The high soil moisture content at the high altitude forest stand can be attributed to a combination of the higher infiltration rate allowing more water into the profile in the high altitude soil, as well as the water extraction by the plants.

The lower moisture content in the low altitude forest soil was the result of quick runoff from the slopes and low water retention capacity of the soil. Much variations in soil moisture content were observed depth wise, however, in most cases it was found to be higher at surface layer than at sub- sub- surface layers. The high acidic nature of the high altitude forest stand may be due to the thickness of the forest. The high organic carbon content of soil at the high altitude may be due to the continuous deposition of eroded soil rich in organic matter brought about by the surface run off of water from the adjacent hill areas. The higher concentration of nutrients in surface (0- 10 cm) soils might be due to higher organic matter content in the surface layer. Surface layer is continuously enriched by the nutrients released from decomposing litters. In addition to regulating the oxygen content of the soil, moisture partly regulates the availability and movement of nutrients to the microbes. Higher concentration of available phosphorus in the soil at the high altitude forest stand could be related to higher microbial activity. Tiwari (1991) suggested that the monthly variation may be related to the rapid release of this nutrient from the litter at the same period. The concentration of available phosphorus remained more or less constant at the deeper soils. Phosphate is relatively immobile and this may be the reason for the little variations noted along the depths. The increased potassium and nitrogen content of soil at the high altitude forest stand may be due to the continuous deposition of eroded soil

rich in organic matter brought about by the surface run off of water from the adjacent hill areas.

Conclusion

From our study, it can be concluded that soil fungal and bacterial CFUs are highly influenced by altitudinal differences and by its biochemical and physico- chemical properties.

Acknowledgement

The authors thank the Head, Department of Botany, North-Eastern Hill University, Shillong, India, for providing the necessary laboratory facilities. The first author is deeply grateful to University Grants Commission- Rajiv Gandhi National Fellowship (RGNF) for SC/ST, New Delhi, for giving financial support to undertake this investigation.

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