Soil CO_2 flux in the different ecosystems of North East India

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We examined monthly and seasonal changes in soil CO_2 flux in the grassland, bamboo and *Dipterocarpus* forest ecosystems of Manipur, North East India. Soil CO_2 flux was recorded to be highest during rainy season and lowest during cool and dry winter season. Soil CO_2 flux rate was recorded to be highest in forest followed by grassland and bamboo forest. Multiple regression revealed that up to 92% of variation in soil CO_2 flux could be explained by soil moisture, soil temperature and soil organic carbon in three different ecosystems. Annual amount of CO_2 flux from soil was estimated at 694.86, 671.16 and 1029.25 g C m^{-2} y^{-1} in grassland, bamboo and *Dipterocarpus* forest ecosystems respectively. Thus the results indicate that soil CO_2 flux rate is highly influenced by seasons, environmental factors and types of vegetation in the different ecosystems of NE India.

Keywords: Multiple regression, soil CO_2 flux, soil moisture, soil organic carbon, soil temperature.

SOIL is a major biosphere reservoir for carbon containing globally twice as much as the atmosphere and three times as much as vegetation[1]. Soil CO_2 emission is the second largest terrestrial carbon flux and is attributed to climate, vegetation type and soil properties. With the increase in atmospheric greenhouse gases and global climate change, soil can either be a net source or a net sink of CO_2 in the future. This depends on which CO_2 flux prevails – the input of carbon into the soil due to plant growth, or the output of carbon through soil respiration.

Soil CO_2 flux is comprised of two major carbon fluxes, i.e. autotrophic respiration of plants root and heterotrophic respiration through the soil microbial activities and is influenced by multitudes of environmental factors[2]. It provides the main carbon efflux from terrestrial ecosystems to the atmosphere and is therefore an important component of the global carbon cycle balance[3,4].

Small changes in soil CO_2 flux across large areas can produce a large effect on CO_2 atmospheric concentration and provide a potential positive feedback between increasing temperature and enhanced soil respiration that may ultimately accelerate global warming[4]. Therefore, detailed information on soil CO_2 flux and its controlling factors is critical for constraining the ecosystem C-budget and for understanding the response of soils to changing land use and global climate change[1,3].

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Soil CO_2 flux can be characterized by its magnitude and its temporal and spatial availability in a specific ecosystem. The rate of soil CO_2 flux is controlled primarily by the rate of CO_2 production by biota within the soil, but is regulated by various biotic and abiotic factors influencing CO_2 movement out of the soil[5]. Generally, soil temperature and soil moisture are considered the most influential environmental factors controlling soil CO_2 flux in the different ecosystems[6–12]. These factors interact to affect the productivity of terrestrial ecosystems and the decomposition rate of soil organic matter, thereby driving the temporal variation of soil CO_2 flux. The heterogeneity of vegetation coverage, root distribution, major environmental factors and soil organic carbon contributes to the spatial variation of soil CO_2 flux in the different ecosystems[13–15].

Soil CO_2 efflux differs among ecosystems and also varies with environmental conditions. Several studies on soil CO_2 flux have been reported in different ecosystems of the world – temperate forest[16,17], tropical forest[18,19], agricultural ecosystem[1], subtropical montane forest[20], subtropical forest[8,21], Mediterranean ecosystems[22], steppe semi-arid ecosystem[23], boreal forest[24], tropical savannas[25], mixed forest[12], bamboo forest ecosystems[26–28], grassland ecosystem[29,30] and *Dipterocarpus* forest[31]. There is lack of information on soil CO_2 flux rate under the different land-use patterns in NE India and South Asia. Therefore, we examined the soil CO_2 flux in three different ecosystems, i.e. grassland, bamboo and *Dipterocarpus* forest in NE India and its response to biotic and abiotic factors.

The major objectives of the present study were to estimate: (i) the monthly and seasonal changes in soil CO_2 flux rate; (ii) variation in soil CO_2 flux among different ecosystems; (iii) effect of soil variables on soil CO_2 fluxes, and (iv) annual soil CO_2 flux rate in grassland, bamboo and tropical forest ecosystems in Manipur, NE India from January to December 2012.

The study site of bamboo forest is located at 24°18′12.5″ N and 94°15′52.9″E at Sibong Khuthengthabi 105 km from Imphal city in Chandel district, Manipur near Myanmar border; the grassland site is located at 24°54′50.5″N and 94°06′16.8″E at Shabungkhok Khunou around 20 km from Imphal city in Imphal East district, Manipur and tropical forest site is located at 24°22′N and 94°29′E near Myanmar border in Chandel district, Manipur about 110 km from Imphal city. Bamboo forest site is dominated by *Schizostachyum pergracile* and grassland site is dominated by *Imperata cylindrica*. The vegetation of tree forest is dominated by *Dipterocarpus tuberculatus.* The soil properties in the three ecosystems are shown in Table 1.

The study area experiences monsoon climate with warm moist summer (March–May) and cool dry winter (November–February), with rainy season during June–October. The mean maximum temperature varied from 22.48°C

Table 1. Physico-chemical properties of soils in bamboo, grassland and *Dipterocarpus* forest ecosystems of Manipur, North East India (mean ± SE)

Parameter	Grassland site	Bamboo forest	*Dipterocarpus* forest site
Soil texture			
Sand (%)	34.57 ± 0.09	39.74 ± 0.13	70.26 ± 0.82
Clay (%)	32.28 ± 0.10	37.17 ± 0.12	17.20 ± 0.23
Silt (%)	33.15 ± 0.08	23.09 ± 0.11	12.54 ± 0.59
Soil pH	5.6 ± 0.23	5.7 ± 0.10	5.7 ± 0.16
Soil temperature (°C)	27.77 ± 0.18	21.23 ± 0.15	24.60 ± 1.15
Soil moisture (%)	13.83 ± 0.62	14.50 ± 0.9	17.41 ± 0.38
Organic carbon (%)	1.86 ± 0.10	1.60 ± 0.15	1.52 ± 0.10
Bulk density (g^{-1} cm^{-3})	1.25 ± 0.16	1.32 ± 0.15	1.20 ± 0.20
Total soil nitrogen (%)	0.24 ± 0.05	0.25 ± 0.01	0.28 ± 0.01
Available soil phosphorus (%)	0.22 ± 0.05	0.31 ± 0.08	0.35 ± 0.05
C : N ratio (%)	1.86 : 0.24	1.60 : 0.25	1.52 : 0.28
Annual litter fall (gm^{-2} $year^{-1}$)	736.25	683.83	620.00

(December) to 30.19°C (May) and the mean minimum temperature ranged from 4.97°C (January) to 22.94°C (August). Annual rainfall was 1166.80 mm and 65% of this was received in rainy season. The mean monthly rainfall ranged from 15.35 mm (December) to 200.66 mm (June). The average relative humidity of air varied between 72.91% (March) and 85.97% (July).

Soil samples were collected from three study sites, i.e. bamboo, grassland and forest randomly at monthly intervals from January 2012 to December 2012 for analysis of physico-chemical characteristics. Soil texture was determined by soil hydrometer (15zH5/60 g/l 68F Zeal). Soil pH was measured by a pH meter in 1 : 5 ratio of soil : water suspension. Bulk density was determined by dividing the dry weight soil sample by its volume and soil moisture content was determined by gravimetric method (oven dry at 105°C for 24 h).

The soil organic carbon was estimated by Walkley–Black method[32]. Total soil nitrogen was measured using 2100 Kjeltec system and available soil phosphorus was determined following the method given by Bray and Kurtz (1945). Five replicates were undertaken for analysis from each site.

Leaf litter was collected at monthly intervals throughout the study by laying 10 trays of 1 m × 1 m size at each study site. Thereafter, it was oven-dried and weighed. Annual carbon input was estimated assuming carbon content of dry weigh to be roughly equal to 50% of annual litterfall.

Soil CO_2 flux was measured by alkali absorption method[33]. Open-ended aluminium cylinders, 13 cm diameter and 25 cm height, were inserted into the soil up to 15 cm depth. The surface area enclosed in each experimental cylinder was 132.7 cm^2. Five cylinders each of the same size were used in each of the study sites, i.e. bamboo, grassland and tree forest, of which one cylinder each was used as blank in all the three study sites. Next 50 ml of 0.25 N NaOH solution was kept in 100 ml plastic vials and the cylinder was made airtight with anchor grip and placed for 24 h to absorb the CO_2 released. The carbon dioxide absorbed was then determined by titrating the NaOH solution with 0.25 N standard dilute HCl solution using phenolphthalein as an indicator. Carbon dioxide absorbed from the soil was calculated using the following formula

$$\text{mg } CO_2 = V \times N \times 22,$$

where V is the volume of the acid and N is the normality of the acid.

All the statistical analyses were carried out using the software, IBM SPSS 20. ANOVA was used to determine the differences in soil CO_2 flux in different months and seasons of the year. Multiple regressions were used to find out relationship between soil CO_2 flux rate and abiotic and biotic factors.

Soil CO_2 flux was between 124.33 ± 1.33 and 586.03 ± 6.39 mg CO_2 m^{-2} h^{-1} in grassland and 163.49 ± 1.50 and 382.13 ± 1.55 mg CO_2 m^{-2} h^{-1} in bamboo site; in forest site it ranged from 158.50 ± 0.42 to 504.90 ± 1.52 mg CO_2 m^{-2} h^{-1} in different months throughout the year (Figure 1). Soil CO_2 flux attained peak value in August/September during the rainy period and was quite low in the dry and cool winter season.

ANOVA indicated a significant difference in soil CO_2 flux between the sampling months in summer ($P < 0.01$), rainy season ($P < 0.01$), winter ($P < 0.01$) and annually ($P < 0.01$).

Soil CO_2 flux showed remarkable seasonal variation in all the three study sites. It was highest during rainy season followed by summer and minimum in winter in the three different ecosystems, which may be due to similar climatic condition prevailing in the region in all the study sites (Table 2). Maximum soil CO_2 flux during rainy season results from spurt mineralization of the labile soil organic matter that has accumulated during the dry period

Table 2. Seasonal variation in soil CO_2 flux in all three study sites (mg CO_2 m^{-2} h^{-1})

Season	Grassland site	Bamboo forest	*Dipterocarpus* forest
Summer (March–May)	248.06 ± 16.81	270.75 ± 5.05	353.86 ± 47.88
Rainy (June–October)	427.25 ± 33.66	334.53 ± 11.78	562.69 ± 9.84
Winter (November–February)	153.22 ± 11.57	222.30 ± 10.84	318.57 ± 3.82
Annual	291.11 ± 11.96	281.18 ± 4.99	429.11 ± 11.25

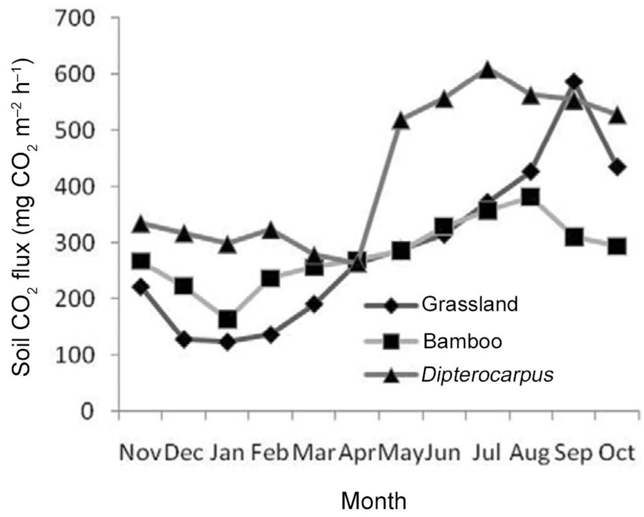

Figure 1. Monthly variation of soil CO_2 flux in the three different ecosystems.

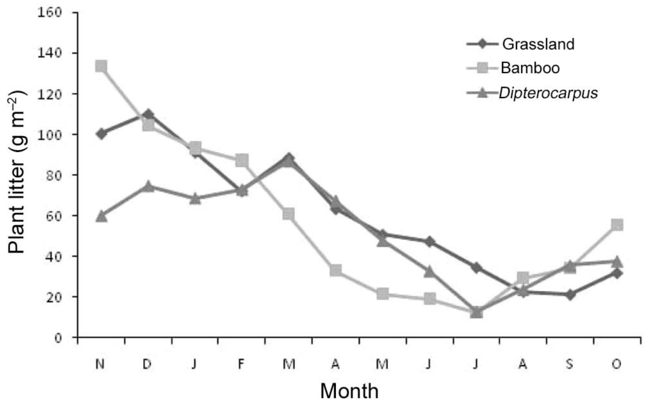

Figure 2. Monthly variation in litter fall in the grassland, bamboo and forest sites.

and which is available to microorganisms after rewetting of the soil[8]. The moist soil condition promoted the physiological activities of soil microbes and root respiration resulted in the increase of soil CO_2 flux. A similar seasonal trend has also been reported in temperate forest[16], subtropical forest[8], warm temperate forest[34], tropical rainforest[15], mixed forests[12,19], Afromontane forest[9] and bamboo plantation[35].

The mean monthly soil CO_2 flux was recorded to be 281.18 ± 4.99, 291.11 ± 11.96 and 429.11 ± 11.25 in bamboo, grassland and *Dipterocarpus* forest respectively. The amount of CO_2 released from soil into the atmosphere was highest in tropical forest followed by grassland and bamboo sites. This may be attributed to the high microbial activities and soil organic matter which was highest in tropical forest followed by grassland and bamboo ecosystem. Besides this, soil nutrient (N and P) was also higher in *Dipterocarpus* forest than grassland and bamboo due to faster rate of litter decomposition in the broadleaved forests than that of grassland and bamboo litter. Thus higher soil resources in the soil component also resulted in greater rate of soil CO_2 flux in *Dipterocarpus* forest. Thus the study shows that broadleaved *Dipterocarpus* forest soil contributes more CO_2 emission to the atmosphere than those of grassland and bamboo vegetation. High rate of soil CO_2 flux in broadleaved forest is also reported in China[21], compared to other types of forests.

A comparative account of soil CO_2 flux in different ecosystems of world is given in Table 3. The present rate of soil CO_2 flux in bamboo forest is comparable with bamboo stand of Japan[36]. Grassland rates are comparable with grasslands of Kurukshetra, India[37] and present forest soil CO_2 flux rates are comparable to tropical forest of Brazil[38] and tropical forest of Costa Rica[39].

ANOVA shows a significant difference in soil CO_2 flux among the three different ecosystems (Table 4). Therefore, it indicates that land-use pattern has strong influence on the emission of CO_2 from the soil into the atmosphere.

Plant litter ranged from 21.40 to 110.12, 12.42 to 133.49 and 12.63 to 86.76 gm^{-2} in different months throughout the year in grassland, bamboo and forest sites respectively (Figure 2). The litter fall was maximum in cool and dry winter months and minimum in rainy months.

Significant negative relationship between soil CO_2 flux and litter fall in different months in all the ecosystems (Figure 3) shows that CO_2 emission from soil was low during cool and dry winter period, coinciding with low microbial activities in spite of heavy litter fall in winter compared to rainy season. Therefore, it shows that soil CO_2 flux is insensitive to the amount of plant litter fall in different ecosystems.

Soil temperature and soil organic carbon were highest in *Dipterocarpus* forest followed by grassland and bamboo, though soil moisture was highest in bamboo followed by forest and grassland sites (Figure 4).

Table 3. Soil CO_2 flux rate in different ecosystems of the world

Vegetation/location	Soil CO_2 flux (mg CO_2 m^{-2} h^{-1})	Reference
Bamboo stand/Japan	166–1295	36
Mixed grassland ecosystem/Canada	0–433	29
Tropical grassland/India	44–448	37
Pasture/Brazil	183–1162	38
Dry dipterocarp forest/Thailand	200–700	31
Warm temperate forest/India	368.00–634.23	16
Tropical forest/Brazil	216–510	38
Tropical forest/Costa Rica	430–675	39
Tropical(primary forest)/Malaysia	948	18
Tropical(primary forest)/Malaysia	707	18
Subtropical forest/ Manipur, India	138.49–250.94	8
Bamboo forest/Manipur, India	163.49–382.13	Present study
Grassland/Manipur, India	124.33–586.03	Present study
Dipterocarpus/Manipur, India	158.50–504.90	Present study

Table 4. ANOVA table of soil CO_2 flux for grassland, bamboo and forest sites ($P < 0.01$)

Source of variation	Sum of squares	d*f*	Mean square	*F*	Level of significance
Between sites	492,424.798	2	246,212.399	18.285	$P < 0.01$
Within sites	1,413,884.435	105	13,465.566		
Total	1,906,309.233	107			

Table 5. Annual soil CO_2 flux (g CO_2 m^{-2} $year^{-1}$) in the three ecosystems

Site	Annual soil CO_2 flux
Grassland	2550.12
Bamboo	2463.14
Forest	3759.00

Table 6. Annual organic carbon input as litter fall (g C m^{-2} $year^{-1}$) and soil CO_2 flux rate output (g C m^{-2} $year^{-1}$) in the three ecosystems

Site	Input	Output
Grassland	368.13	694.80
Bamboo	341.50	671.16
Forest	310.00	1029.25

The relationship between rate of soil CO_2 flux (mg CO_2 m^{-2} h^{-1}) and soil properties, i.e. soil temperature (X_1), soil moisture (X_2) and soil organic carbon (X_3) has been analysed by multiple regression in all the study sites and is summarized as follows:

Grassland site

$Y = -683.446 + 1.229X_1 + 3.557X_2 + 498.496X_3$,
($r_1 = 0.81$; $r_2 = 0.68$; $r_3 = 0.97$) at $P < 0.01$.

Bamboo forest site

$Y = -104.406 + 4.301X_1 + 0.393X_2 + 163.729X_3$,
($r_1 = 0.93$; $r_2 = 0.95$; $r_3 = 0.90$) at $P < 0.01$.

Dipterocarpus forest site

$Y = -20.479 + 52.440X_1 + 24.329X_2 - 5.629X_3$,
($r_1 = 0.57$; $r_2 = 0.97$; $r_3 = 0.84$) at $P < 0.01$.

The study shows that significant positive relationship between soil CO_2 flux rates and variables: soil moisture, soil temperature and soil organic carbon has strong influence on the release of CO_2 into the atmosphere from the soil in the three ecosystems. The 68% to 92% variation in soil CO_2 flux rate is explained by these three variables. Similar findings have also been reported in different ecosystems by several workers[2,8,11,17,18,34,40].

Annual soil CO_2 was the cumulative soil CO_2 flux rate from daily mean monthly values (Table 5). Annual soil CO_2 rate in the study sites was highest in *Dipterocarpus* forest followed by grassland and bamboo sites. These values are comparable with the reported data (2936–3694 g CO_2 m^{-2} $year^{-1}$) in deciduous broadleaved forest in northern Japan[41] and higher than the value of 2000 g CO_2 m^{-2} $year^{-1}$ in mixed broadleaf and conifer forest of USA[42] and lower than the value of 3915–4235 g CO_2 m^{-2} $year^{-1}$ of teak plantation in Thailand[43].

Annual carbon input and annual carbon output were estimated by annual soil CO_2 flux rate (g C m^{-2} $year^{-1}$; Table 6). The input and output of CO_2 in the different sites show that the annual output of CO_2 flux is almost three times than the input in *Dipterocarpus* forests and twice in other two sites, i.e. grassland and bamboo (Table 6). The difference between input and output of CO_2 may be due to root respiration rate and CO_2 emission by root decomposition or root exudates or underestimates of litter fall. Besides, litter decomposition rate is higher in broad-leaved *Dipterocarpus* forest than that of grassland, and bamboo is also related to low C/N ratio of forest soil as microbes attack the rich nitrogen resources compared to grassland and bamboo sites. Thus it shows that output of CO_2 is maximum in forest and minimum in bamboo ecosystem. Further studies are needed to analyse the role of microorganisms and the activities of vegetation types for estimation of soil CO_2 flux in different ecosystems.

The study indicates that conversion of forests into grassland or bamboo ecosystem would result in reduction of CO_2 emission from the soil. Thus change in land-use pattern will have long-term implication on the carbon management strategies in the NE India.

Thus the present study shows that seasons have a strong influence on the soil CO_2 flux with highest rate in rainy season and lowest in cool and dry winter season in three ecosystems. Soil temperature, soil moisture and soil organic carbon are the predominant variables controlling soil CO_2 flux in all the three ecosystems. Annual soil CO_2 flux was highest in forest followed by grassland and bamboo ecosystems. It shows that forest soil emits more

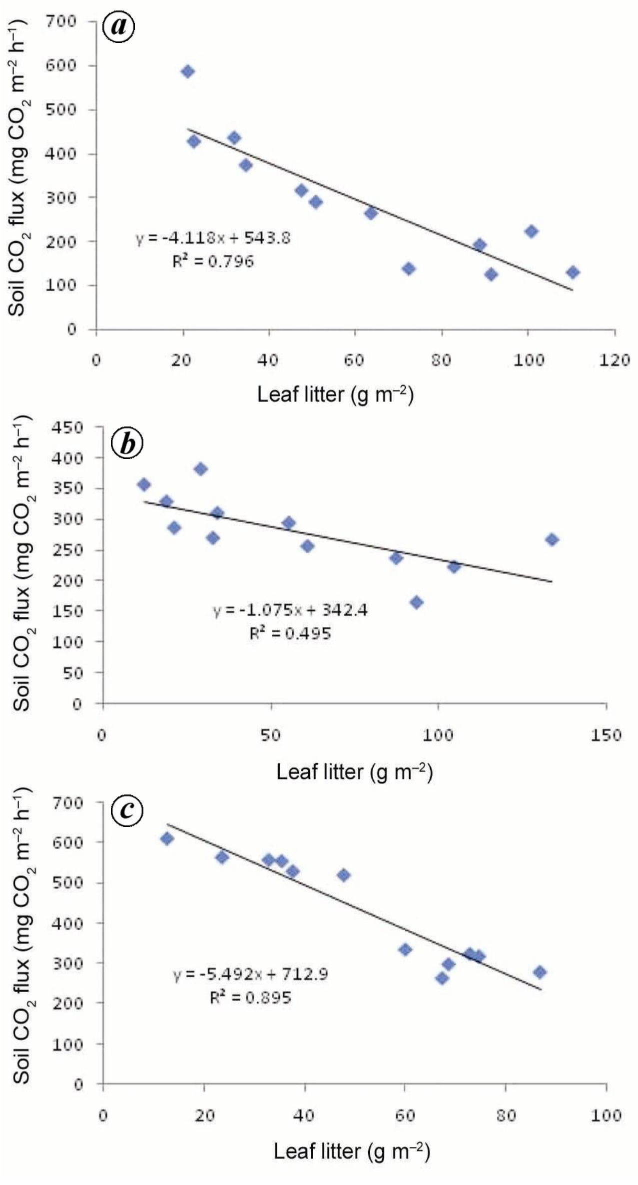

Figure 3. Relationship between soil CO_2 flux and plant litter in different months throughout the year in (***a***) grassland, (***b***) bamboo and (***c***) forest ecosystems.

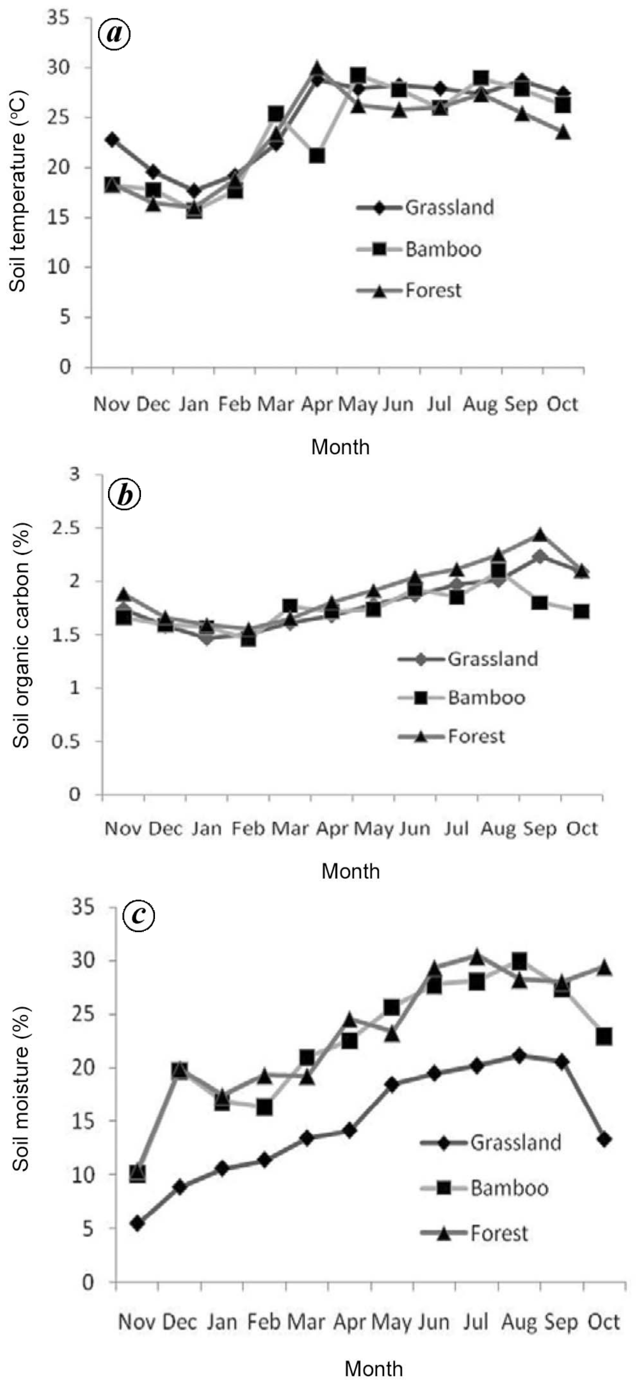

Figure 4. Monthly variation of (***a***) soil temperature, (***b***) soil organic carbon and (***c***) soil moisture in the three different ecosystems.

CO_2 into the atmosphere than that of grassland and bamboo ecosystems. These findings will enable us to accurately estimate regional carbon fluxes and carbon budget by taking into the consideration the biotic and abiotic variables in different ecosystems of NE India.

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Isolation of predominant bacterium from gut of earthworm *Lampito mauritii* for effective use in soil fertility

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***Lampito mauritii* is an anecic earthworm living in the topsoil and it is geophytophagous in nature. This earthworm is an important soil macrofauna as it has the dual role of an 'ecosystem engineer' due to the ability to build burrows as well as 'keystone species' in soil food webs because of its function in degradation of organic wastes. The present study investigates the gut of this earthworm to find the most predominant bacterium harboured therein. Gut contents were regularly extracted and streaked on bacteriological media. The predominant type of colony was identified, isolated and streaked separately to get pure colonies. The microbe was subjected to several biochemical tests and also 16S rRNA sequencing for identification. On the basis of these tests, the bacterium was identified as *Bacillus cereus*. The microbe was used as a composting agent on solid wastes as a result of which good amount of plant nutrients, specially nitrogen (20.3 kg/acre), phosphate (27.4 kg/acre) and potassium (52.1 kg/acre) were found in the resultant manure. The compost thus obtained was then utilized for the production of vegetables with an attempt to protect soil environment, thus reducing the deleterious effects of chemical fertilizers.**

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THE living community of the soil, including both fauna and flora, plays a major role in decomposition, humification and litter formation[1]. Of the innumerable life forms that inhabit the soil, only a small number of macro invertebrates (earthworms, termites and ants) are distinguished by their capacity to excavate the soil and produce a wide variety of organomineral structures, such as excretions, nests, mounds, macropores, galleries and caverns. These organisms have been described as 'ecological engineers' of the soil[2] and their structures as 'biogenic structures'[3]. Earthworms form one of the major soil macrofauna to maintain dynamic equilibrium and regulate soil fertility[4]. The soil volume affected by earthworm activities is called the drilosphere[5], which is a major soil functional domain[6].

Earthworm activity does not only mediate macroaggregate formation, but also microaggregate formation[7,8]. Based on thin sections of the earthworm gut, casts and control soil from earthworm microcosms, several studies have shown that during gut transit organic materials are intimately mixed and become encrusted with the mucus to create new nuclei for microaggregate formation[7–9]. On the other hand, earthworm casts significantly affect plant growth through their effects on microorganisms, aggregation of soil and nutrient supply[10]. Casts have been shown to have enhanced microbial and enzyme activities and micro- and macro-nutrients[11]. Many authors have reported the occurrence of several species of bacteria and fungi in earthworm casts[12,13]. Many cellulolytic, nitrifying and denitrifying bacteria have been observed in earthworm casts[14]. Several workers have found that microorganisms flourish in earthworm casts. Teotia *et al.*[15] reported that earthworm casts had a bacterial count of 32.0 million/g compared to 6.0–9.0 million/g in the surrounding soil. Daniel and Anderson[16] experimented with *Lumbricus rubellus* and observed that the casts in four different soils contained greater number of bacteria than the soils. During formation in the earthworm gut, the 'would be' casts are colonized by microbes that begin to breakdown soil organic matter[17].

According to Julka *et al*[18], in India there are 590 species of earthworms with different ecological preferences. *Lampito mauritii* is the most widely distributed earthworm in