



## Enzyme activity and carbon dioxide evolution from upland and wetland rice soils under three agricultural practices in hilly regions

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**Summary.** Dehydrogenase activity (a measure of microbial biomass), urease activity and CO<sub>2</sub> evolution were measured in soils planted to rice (*Oryza sativa* L.) under three different agricultural practices prevalent in hill regions. The effects of hill slope, terrace and valley agriculture were investigated for two cropping seasons. The valleys and terraces were kept flooded during each cropping season while the hill slopes were cultivated with dryland practices. The type of agricultural practice and the date of observation had a significant influence on enzyme activity and CO<sub>2</sub> evolution. A positive correlation was observed between dehydrogenase and urease activity and soil moisture content. Dehydrogenase and urease activity and soil respiration were positively correlated among themselves. The activity of both enzymes and CO<sub>2</sub> evolution were highest in valley soils followed by terrace and hill-slope sites.

**Key words:** Soil enzymes – Soil respiration – Hill agriculture – Wetland rice – Dehydrogenase activity – Urease activity

Soil is a living system in which biological activities take place with the help of enzymatic processes. Quantitative measurements of soil enzyme activities can contribute to our understanding of these biological transformations by allowing us to evaluate the activity present in the soil. Intracellular dehydrogenases (measure of microbial biomass) are indirectly involved in

the initial stages of the oxidation of soil organic matter since they mediate the transfer of H electrons from substrate to acceptors. Measurement of the activity of intracellular dehydrogenases thus provides information on the biological activities of soil microorganisms (Casida 1977; Dkhar and Mishra 1983; Bolton et al. 1985); it is a useful indicator of microbial metabolism levels and is hence a parameter for the activity of the soil microflora and for microbial biomass (Benckiser et al. 1984).

Urea added to soils as fertilizer is rapidly hydrolysed to ammonium carbonate in most soils through the activity of soil urease; this enzyme is responsible for a rapid release of ammonia when urea is applied (Hoult and McGarity 1986). The growing importance of urease as an N fertilizer in world agriculture has revealed a need for research to reduce the damage done to germinating seedlings and young plants by the release of ammonia and to reduce this form of volatilization of urea-N. Urease is a constitutive enzyme found in a large number of microorganisms, especially in urolytic bacteria and many fungi. Previous research has suggested that urease activity can be used as an indicator for total biological activity and for soil fertility.

The rate of CO<sub>2</sub> release from soil may reflect the general activity of soil microorganisms (Edwards et al. 1981). Soil respiration was one of the earliest and is still one of the most frequently used indices of microbial activity in soils. The study of soil respiration in a given ecosystem may lead to an assessment of the organic matter input into the system, the flow of energy through the system's microbial components and the rate of N mineralization (Macfadyen 1970).

In hilly regions crops are grown on the hill slopes, on terraces constructed on the slopes and on the flat land found in the valleys. In north-eastern hilly regions of India soils under three main types of

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agriculture are subject to varying degrees of erosion caused by the heavy rainfall (Fig. 1). On the hill slopes a primitive type of agriculture (shifting agriculture) is practised; consists of cutting the natural vegetation on the slopes, allowing the slash to dry for a few months and then burning it before cropping. The rice seeds are sown in the last week of May, after a shower of rain,

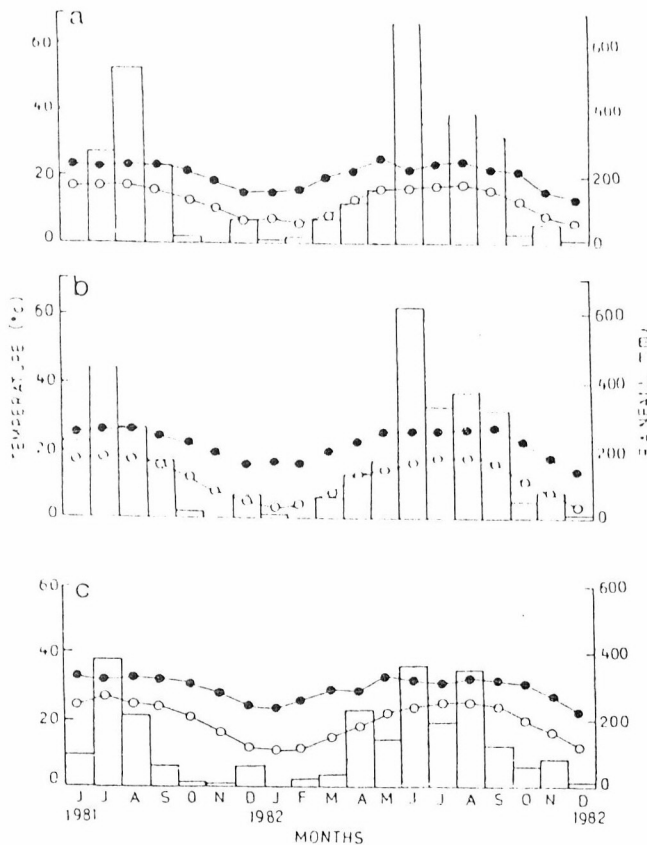


Fig. 1a-c. Mean monthly variations in temperature and rainfall of the study areas. **a** Upper Shillong (terrace); **b** Shillong (valley); **c** Burnihat (hill-slope). Histogram, rainfall; closed circles, maximum temperature; open circles, minimum temperature

by dibbling with a long stick. This type of cultivation is carried out on slopes of 20°–40°. Hill-slope soils therefore suffer maximum soil erosion due to the slope and to the faulty agricultural practice of making all the bands parallel to the slope. A large amount of surface soil is lost with every rain and very much less water percolates. Soils under this type of agriculture lose their fertility very rapidly due to these erosion losses and the farmers abandon the land after 3–4 years of cultivation and move on. Land found between the hillocks is termed valley land, and comprises low lying areas which are flooded for part of the year and contain a high level of moisture. Depending on the topography and the drainage farmers either grow two crops of rice or one crop of rice followed by potatoes. Valley soils are generally fertile due to the natural inflow of nutrients from adjoining hills. These lands are ideally suitable for rice cultivation. In areas where sufficient land is not available for valley or hill-slope agriculture bench terraces are formed on the hillocks. Terracing checks soil erosion and helps in retaining water, allowing the soil to be cultivated for a longer period. The width of the terraces depends on the degree of slope, i.e., the higher the slope angle, the narrower the terrace. Erosion losses are reduced under terrace agriculture and reach a minimum under valley agriculture (Mishra and Ramakrishnan 1983). The terraces are flooded as soon as the rainy season starts and then rice is grown, followed by potatoes. Rice is the principal agricultural crop of the region, being grown on all three types of land. The three agricultural practices have been compared for their economic yield and energy budget (Toky and Ramakrishnan 1981, 1982). No study is available on the biochemical aspects of soils receiving water erosion. The present study therefore investigated dehydrogenase and urease activities and soil respiration under the three agricultural systems found in these hill regions, in order to see how soil microbial activity is affected by the water erosion and allied factors.

Table 1. Soil texture, type of rice, crop rotation and type of manuring in three agricultural systems

	Hill-slope	Terrace	Valley
Soil type	Laterite Sandy loam	Laterite Sandy loam	Laterite Sandy loam
Type of rice	Sowing by dibbling Variety: local Maturity time: 150 days	Sowing by seed broadcast Variety: local Maturity time: 180 days	Sowing by seed broadcast Variety: local Maturity time: 180 days
Crop rotation	Rice – maize – fallow	Rice – fallow or Rice – potato	Rice – rice – fallow
Manuring/fertilizer	Nil	Nil	Nil

## Materials and methods

The investigation was carried out during two rice-cropping cycles (*Oryza sativa* L.) from June 1981 to December 1981 and from June 1982 to December 1982. Wetland rice was grown in valleys and on terraces; the hill-slope rice was a dryland type. Soil and crop rotation details are given in Table 1. The valley site is at Shillong (altitude 1340 m, latitude 25°34'N, longitude 91°50'E), the terrace site at Upper Shillong (altitude 1540 m, latitude 25°34'N, longitude 91°56'E) and the hill-slope site at Burnihat (altitude 100 m, latitude 26°N, longitude 91°50'E). The distance between Shillong and Upper Shillong is 7 km, and between Shillong and Burnihat 80 km. Soil samples were collected from three depths (0–10, 10–20, and 20–30 cm) at about 11 a.m. on every sampling date. Five samples were collected from each site and mixed thoroughly to make a composite sample. Three replicate analyses were performed from each composite sample. All precautions were taken to avoid contamination, and all estimations were carried out within 24 h of collection. The data were statistically analysed using a three way analysis of variance (Snedecor and Cochran 1967).

Dehydrogenase activity was determined by the 2,3,5-tetrazolium chloride reduction technique as suggested by Casida (1977). One

gram of freshly collected soil sample was transferred into a test tube (15 × 1.5 cm) mixed with 0.1 g CaCO<sub>3</sub> and 1 ml of 1% tetrazolium chloride solution. The content was well homogenized, plugged with a rubber stopper, and incubated at 30°C for 24 h. The triphenyl formazan was extracted with methanol and assayed colorimetrically at 485 nm using a Spectronic-20 spectrophotometer.

Urease activity was determined by the method of McGarity and Myers (1967). One gram of soil was placed in a 100-ml volumetric flask and 1 ml toluene was added, then 10 ml of buffer solution (pH 7) and 5 ml of a 10% urea solution were added; the flasks were then shaken and incubated for 3 h at 37°C. Ammonia released as a result of urease activity was determined by the indophenol blue method and assayed colorimetrically at 630 nm. The amount of ammonia-N formed was calculated using a standard curve prepared with an ammonia solution. Three replicates were used in all experiments.

The CO<sub>2</sub> evolution from soil was measured by an absorption and titration method (Macfadyen 1970). A 1-kg fresh soil sample (from which all root materials had been removed) was placed in a glass jar (15 × 15 × 20 cm), and glass beakers (100 ml) containing 50 ml of 0.1 N KOH solution were placed inside the jars. The glass jars were then sealed and made air tight with grease. A control was used to determine the subtraction of atmospheric CO<sub>2</sub>. After 24 h of incubation at room temperature (20–25°C) amount of CO<sub>2</sub> fixed by the KOH solution was determined by a titrimetric method using 0.1 N HCl and phenolphthalein as an indicator. Finally, the CO<sub>2</sub> evolved was expressed in mg CO<sub>2</sub> evolved per kg dry soil.

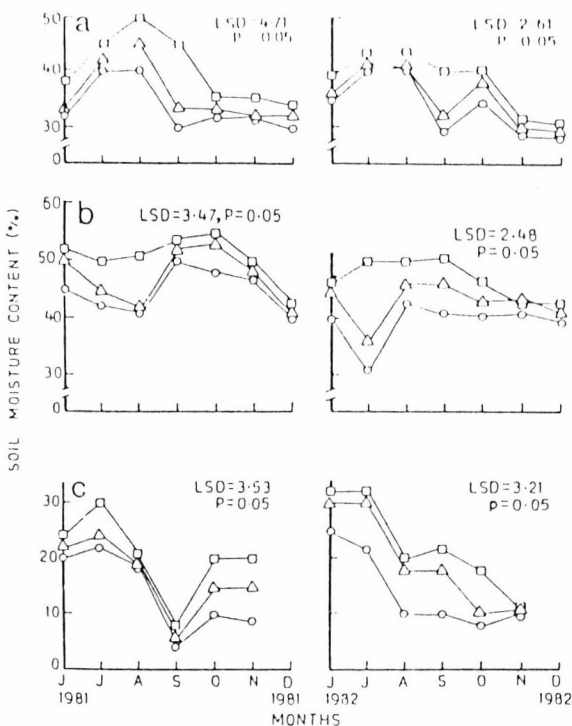


Fig. 2a–c. Monthly variations in moisture content (%) in soils of terrace a valley, b and hill-slope, c rice fields. Circles 0–10 cm depth; triangles, 10–20 cm depth; squares, 20–30 cm depth

## Results

The percentages of N and organic C in the soils under the three agricultural practices are given in Table 2. The valley soils contained the most organic C and N while the hill slopes contained the least. Soil moisture data are given in Fig. 2. The valley and terrace soils contained high moisture levels throughout the study period while the hill-slope soils contained much less. The flooding took place in July–August in the terrace site and until September in the valley site.

Monthly variations in dehydrogenase activity are shown in Fig. 3. The highest level of activity was recorded in the valley soils. In the terrace soils the highest level of dehydrogenase activity was observed in August in both years, while in the valley soils dehydrogenase activity reached a maximum in the month of September or October and thereafter declined. The lowest activity levels were generally recorded at sowing (June) and harvest (December). The general trend of the monthly variation was similar in both years, with

Table 2. Organic C and N of the soils of three agricultural systems. Values are means of 14 monthly determinations in two crop cycles

	Hill-slope soils			Terrace soils			Valley soils		
	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20–30 cm	0–10 cm	10–20 cm	20–30 cm
Organic C (%)	0.85	0.61	0.20	1.33	0.89	0.75	1.68	1.61	1.10
N (%)	0.12	0.08	0.06	0.21	0.17	0.13	0.22	0.17	0.14

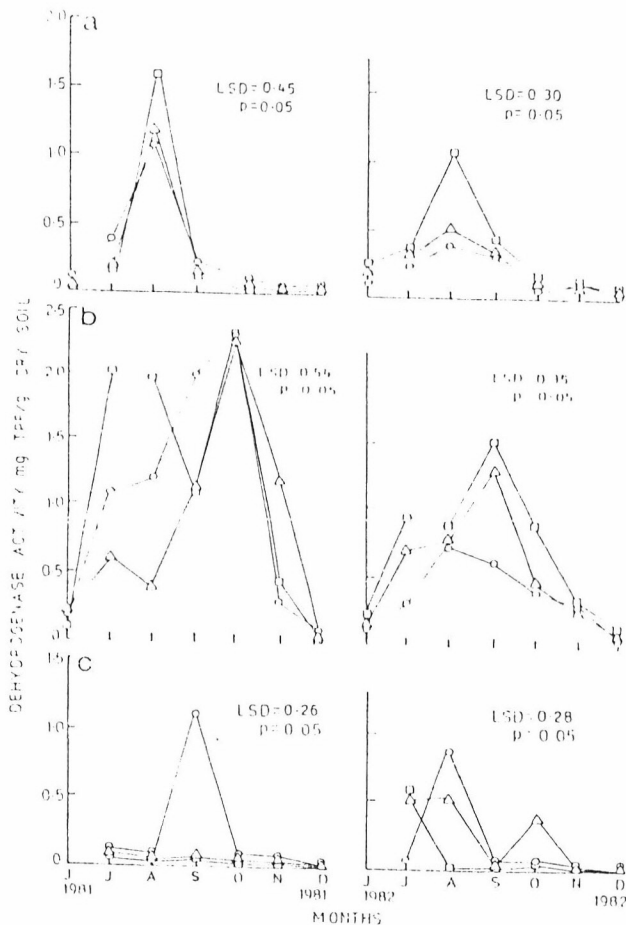


Fig. 3a-c. Monthly variations in dehydrogenase activity in soils of a terrace, valley; b and hill-slope; c rice fields. Soil depths as shown for Fig. 2

the hill-slope soils showing generally less dehydrogenase activity than the other soils. In the wetland soils there was a gradual seasonal build-up of activity, followed by a decline in the winter. However, in the dryland soils, except for one or two peaks dehydrogenase activity generally remained very low.

Figure 4 shows the urease activity of the three sites. In the terrace soils the highest level of urease activity was observed in August-September. In the valley soils, for both years, the highest urease activity was noticed in September. The activity was less in the post-harvest month of December. At most samplings no significant difference was observed in urease activity among the three depths. In the hill-slope soils, urease activity was higher in August during the 1st year and in September-October during the 2nd year. In 1982, during the first 3 months, urease activity was higher in the deeper layer (20-30 cm depth) but later high values were observed in the surface layer (0-10 cm depth).

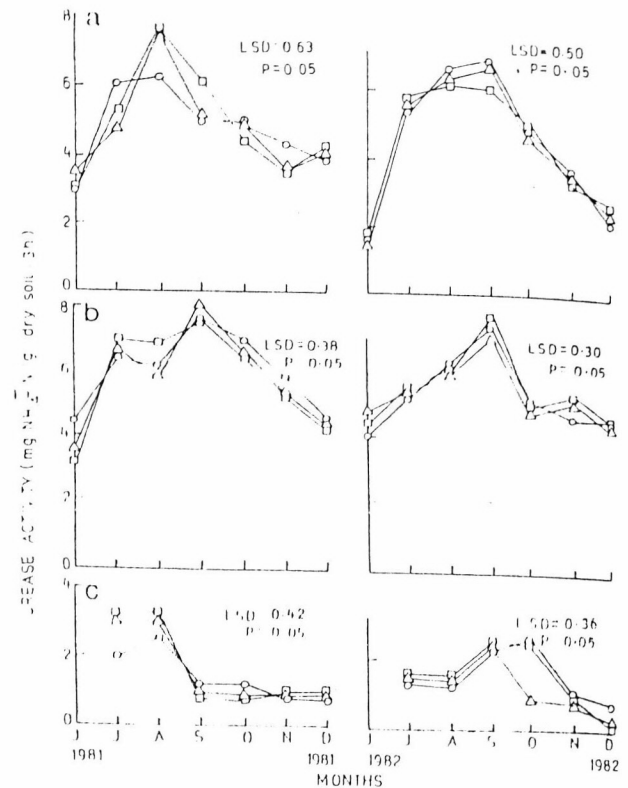


Fig. 4a-c. Monthly variations in urease activity in soils of terrace a valley; b and hill-slope; c rice fields. Soil depths as shown for Fig. 2

CO<sub>2</sub> evolution from soils of the different rice-growing areas is shown in Fig. 5. In the terrace soils CO<sub>2</sub> evolution was highest in July and August. Surface soils (0-10 cm) showed higher levels of CO<sub>2</sub> evolution than the deeper layers. In the valley soils, the highest level of CO<sub>2</sub> evolution was observed in September. Generally, a higher level of CO<sub>2</sub> evolution was recorded from the surface soils, and values for soils at 20-30 cm depth were consistently lower. The hill-slope soils showed less CO<sub>2</sub> evolution than the other soils.

A three-way analysis of variance showed that the type of agricultural practice and the date of observation had a significant influence on enzyme activity ( $P = 0.05$ ). Simple correlation coefficient ( $r$ ) values among various soil characteristics for the various soils are given in Table 3. Most of the biological parameters are positively correlated with each other. Moisture and temperature also significantly influenced the microbial activity of the rice-field soils.

## Discussion

Dehydrogenase activity was very low under hill-slope (dryland rice) agriculture, but was higher in the valley

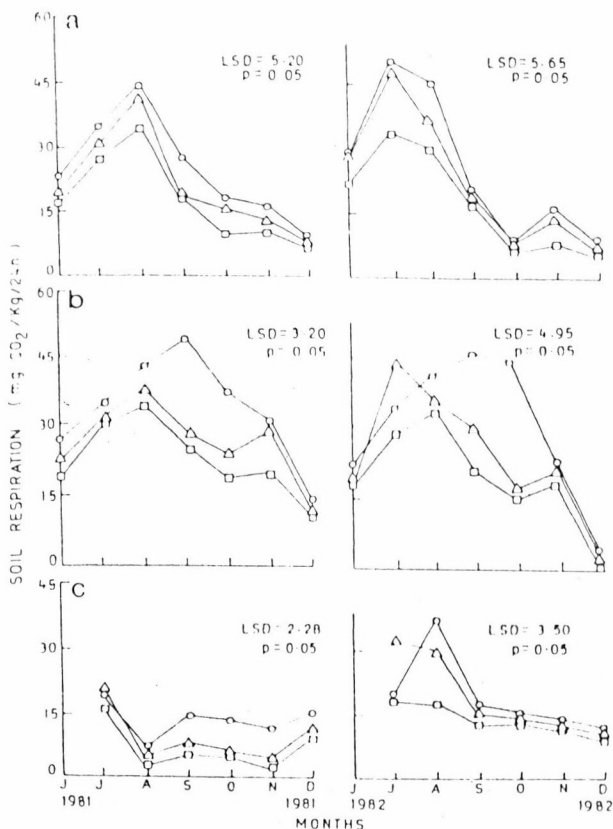


Fig. 5a–c. Monthly variations in soil respiration in soils of terrace valley; b and hill-slope; c rice fields. Soil depths as shown for Fig. 2

Table 3. Simple correlation coefficient ( $r$ ) values among various soil characteristics of rice fields. Computation is based on data from three types of agricultural practice and three different soil depths

	Dehydrogenase activity	Urease activity	CO <sub>2</sub> evolution
Temperature	0.61*	0.55*	0.77**
Moisture content	0.79**	0.73**	0.86**
N	0.75	0.47*	0.92**
Dehydrogenase activity		0.71**	0.91**
Urease activity			0.73**

\*  $p = 0.05$ ; \*\*  $p = 0.01$

and terrace soils (Fig. 3), indicating that metabolic activity of the microflora was higher in the flooded soils. In the upland soils, the lower soil moisture and organic-C contents may have been unfavourable to microbial activity. In earlier studies we reported that field-capacity moisture (48%) allowed optimal dehydrogenase activity (Tiwari et al. 1987a) and a direct positive relationship ( $r = 0.79$ ) between dehydrogenase activity and soil moisture has been reported (Baruah

and Mishra 1984). The valley soils are relatively rich in inorganic nutrients and organic C (Table 1; Mishra and Ramakrishnan 1983) and this may have contributed to the higher dehydrogenase activity in these soils. Earlier, Tate and Terry (1980) and Chendrayan et al. (1980) had also reported higher dehydrogenase activity in flooded rice soils, which they ascribed to facultative anaerobic bacteria. There is evidence that dehydrogenase activity is affected by the presence of ferrous Fe (Benckiser et al. 1984). In flooded anaerobic soils Fe<sup>3+</sup> compounds become rapidly reduced to Fe<sup>2+</sup> compounds by direct enzymatic reduction (Munch and Ottow 1983). The high levels of dehydrogenase activity at depth and the longer duration of the activity in the valley soils may indicate that flooding and formation of Fe<sup>2+</sup> are related to dehydrogenase activity.

Urease activity was higher in the terrace and valley soils (wetland agriculture) than the hill-slope soils (dryland agriculture), probably due to the higher soil moisture and organic-C content of the wetland rice soils compared with the dryland soils (Rao and Ghai 1985). There was a positive relationship between urease activity and moisture content, temperature, nitrogen, and dehydrogenase activity (Table 3). A similar relationship was noted in an earlier study conducted in pineapple orchard soils of this region (Tiwari et al. 1987b). Dkhar and Mishra (1983), Sahrawat (1983), and Rao and Ghai (1985) have reported that urease activity is positively related to soil organic-C content but this was not a finding of the present study. Temporal variations in urease activity may be related to variations in soil temperature and moisture, as most rain occurs between June and September and the months October–December are generally cold and dry (Fig. 1).

CO<sub>2</sub> evolution was highest in the surface layer (0–10 cm) and decreased with increasing depth (Fig. 5) due mainly to the decreasing organic-matter content (Schinner et al. 1980). The high level of organic matter in surface soils seems to be most important factor in the higher CO<sub>2</sub> evolution, but cannot explain much of the variance. Correlations of respiratory activities with organic-C content and with total N appear largely due to the common influence of other factors. The present results show that CO<sub>2</sub> evolution was related to soil temperature and moisture, as soil respiration was at its lowest during the low-temperature conditions in November and when the moisture content of the soil was also low, thus supporting the findings of Anderson (1973). In the present study CO<sub>2</sub> evolution was significantly correlated ( $P = 0.05$ ) with soil moisture ( $r = 0.86$ ), nitrogen ( $r = 0.92$ ), temperature ( $r = 0.77$ ), and dehydrogenase ( $r = 0.91$ ) and urease activity ( $r = 0.73$ ).

Figures 2–5 show that the onset of the rainy season and the increase in soil moisture content triggered microbial activity. The flooding of the soils (terrace and valley) also had a pronounced effect on enzyme activity and soil respiration, since the peaks of the activities were earlier in terrace and delayed in the valley soils, corresponding to the times of flooding in these two sites. In the flooded paddy-field soils of the valley the enzyme activities were greater than in the terrace and hill-slope soils. Wetland rice soils thus exhibited a higher rate of enzyme activity than dryland rice soils. This can be ascribed mainly to the higher nutrient contents and higher levels of soil moisture in the wetland soils (Mishra and Ramakrishnan 1983). From the present measurements of dehydrogenase and urease activity and the rate of CO<sub>2</sub> evolution we conclude that valley soils support the highest level of microbial activity and hill-slope soils the lowest, while terrace soils fall between the two.

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## References

- Anderson JM (1973) CO<sub>2</sub> evolution from temperature deciduous woodland soils. *J Appl Ecol* 10:361–378
- Baruah M, Mishra RR (1984) Dehydrogenase and urease activities in rice field soils. *Soil Biol Biochem* 16:423–424
- Benckiser G, Santiago S, Neue HE, Watanabe I, Ottow JCW (1984) Effect of fertilization on exudation, dehydrogenase activity, iron-reducing populations and Fe<sup>2+</sup> formation in the rhizosphere of rice (*Oryza sativa* L.) in relation to iron toxicity. *Plant and Soil* 79:305–316
- Bolton H Jr, Elliott LF, Papendick RI, Bezdicke DF (1985) Soil microbial biomass and selected soil enzyme activities: Effect of fertilization and cropping practices. *Soil Biol Biochem* 17:297–302
- Casida LE Jr (1977) Microbial metabolic activity in soil as measured by dehydrogenase determinations. *Appl Environ Microbiol* 34:630–636
- Chendrayan K, Adhya TK, Sethunathan N (1980) Dehydrogenase and invertase activities of flooded soils. *Soil Biol Biochem* 12:271–273
- Dkhar MS, Mishra RR (1983) Dehydrogenase and urease activities of maize (*Zea mays* L.) field soils. *Plant and Soil* 70:327–333
- Edwards NT, Stugart HH Jr, McLaughlin SB, Harris WF, Reichle DE (1981) Carbon metabolism in terrestrial ecosystems. In: Reichle DE (ed) *Dynamic properties of forest Ecosystems*. IBP 23, Cambridge University Press
- Hoult EH, McGarity JW (1986) The measurement and distribution of urease activity in a pasture system *Plant and Soil* 93:359–366
- Macfadyen A (1970) Soil metabolism in relation to ecosystem energy flow and to primary and secondary production. In: Phillipson J (ed) *Methods of study in soil ecology*. Proceedings of the Paris Symposium, UNESCO, International Biological Programme, pp 167–172
- McGarity JW, Myers MG (1967) A survey of urease activity in soils of northern New South Wales. *Plant and Soil* 27:217–238
- Mishra BK, Ramakrishnan PS (1983) Slash and burn agriculture at higher elevations in north-eastern India: I Sediment, water and nutrient losses. *Agric Ecosyst Environ* 9:69–82
- Munch JC, Ottow JCG (1983) Reductive transformation mechanism of ferrous oxides in hydromorphic soils. *Ecol Bull (Stockh)* 35:383–394
- Rao DLN, Ghai SK (1985) Urease and dehydrogenase activity of alkali and reclaimed soils. *Aust J Soil Res* 23:661–665
- Sahrawat KL (1983) Relationships between soil urease activity and other properties of some tropical wetland rice soils. *Fert Res* 4:145–150
- Schinner FA, Neederbacher R, Neuwinger T (1980) Influence of compound fertilizer and cupric sulfate on soil enzymes and CO<sub>2</sub> evolution. *Plant and Soil* 57:85–93
- Snedecor GW, Cochran WG (1967) *Statistical methods*, 6th ed. Iowa State University Press, Ames
- Tiwari SC, Tiwari BK, Mishra RR (1987a) The influence of moisture regimes on the population and activity of soil microorganisms. *Plant and Soil* 101:133–136
- Tiwari SC, Tiwari BK, Mishra RR (1987b) Temporal and depthwise variations in dehydrogenase and urease activities and bacterial population in pineapple plantation soils. *Proc Indian Natl Sci Acad B* 53:173–176
- Tate RL, Terry RE (1980) Variation in microbial activity in Histosols and its relationship to soil moisture. *Appl Environ Microbiol* 40:313–317
- Toky OP, Ramakrishnan PS (1981) Cropping and yields in agricultural systems of north-eastern hill region of India. *Agro Ecosyst* 7:11–25
- Toky OP, Ramakrishnan PS (1982) A comparative study of the energy budget of hill agro-ecosystem with emphasis on the slash and burn system (Jhum) at lower elevations of north-eastern India. *Agric Syst* 9:143–145

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