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Soil Urease Activity of Sundarban Mangrove Ecosystem, India

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Abstract

Vertical occurrence of soil urease activity along with ammonia content from three distinct regions viz. Deep forest region (No tidal action and wave attack occurs as it is furthest from river shore and it contains maximum content of organic carbon and minimum soil salinity and silicate concentration. In this zone plenty of pneumatophores, below ground root and dense vegetation are found), Rooted region (It is situated in between Deep forest region and Un-rooted region. This region contains only pneumatophores but it is devoid of long roots and vegetations. It faces wave attack and tidal action less than that of Un-rooted region) and Un-rooted region (It is closest to river shore and faces maximum wave attack and tidal action; it contains minimum organic carbon but maximum soil salinity and silicate concentration. This zone is totally devoid of any roots, pneumatophores and vegetations) of Sundarban mangrove forest ecosystem, India revealed an interesting explanation. Soil urease activity showed a decreasing pattern with increase in depth from the deep forest region of the Sundarban forest ecosystem. Soil urease activity was found to be more sensitive to soil temperature and pH rather than soil salinity. This ensured that soil urease along with the microbes present in the Sundarban forest ecosystem are more tolerant to fluctuation in salinity than that of temperature. Soil ammonia concentration was found to be directly governed by the soil urease activity [The regression equation is Ammonia in soil = −1.64 + 0.0402 Urease Activity (R-Sq = 62.9%, $P < 0.001$, $n = 41$)].

Keywords

Soil Urease Activity, Soil Ammonia, Deep Forest Region, Rooted Region, Un-Rooted Region, and Sundarban Forest Ecosystem

1. Introduction

Mangroves accounting for 0.7% of the tropical forest area are highly productive [1] [2] [3] and afford territory for rich biodiversity [4]. The vast majority of the nutrient pool of mangrove forests is stored in the soil and many mangrove soils have extremely low nutrient availability [5]. Rapid recycling of nutrient from litter is essential to maintain high productivity and microbial processes are more important over chemical processes in the sediment. Nutrient dynamics along with soil enzyme activity of Sundarban mangrove forest is highly influenced by periodic seasonal change, sedimentation rate, water level, leaf fall, etc. In a tropical mangrove forest of Brazil significant sediment accumulation rate was observed to control nutrient status of mangrove sediment [6]. Previous research works reported that mangrove sediments may act as a vast reservoir of organic matter as it initiates the production and rapid burial of the organic matters and it was influenced by sea level rise [7].

Urease (urea amidohydrolase, EC 3.5.1.5) is an important enzyme in soil because of the hydrolytic action on urea that may be excreted in the urine of grazing animals, but its origins, existence and persistence in soil are difficult to understand. The reaction occurs as follows:

$$(NH_2)_2CO(Urea) + H_2O \rightarrow CO_2\uparrow + 2NH_3\uparrow$$

It is generally believed that a significant proportion of urease activity in soil is released from living and lysed microbial cells and is stabilized as an extra cellular enzyme by association with soil colloids [8], especially soil organic matter [9]. Soil urease activity along with microbial activity was found to be significantly affected by physical disturbance in soil and also by the geochemical parameters [10]. Mangrove ecosystem has unique characteristics to adapt with stressful environments and it has a huge demand for nutrients because of rapid growth of mangrove plants and for fulfillment of such demands the mangrove soil act as a paradise land for several soil enzymes like urease to facilitate easy and quick bio-mineralization processes [11]. Total urease activity in soil may comprise activities associated with viable microorganisms, clay and humic colloids, leaked from extant cells, or released from lysed cells and cell debris. However, urease which becomes associated with humic colloids due to adsorption, entrapment or copolymerization during organic matter formation would persist for a long period [12]. Urease is principally an extra cellular enzyme, representing up to 63% of total urease activity in soil [13]. Significant occurrence of soil urease activity facilitates biomineralization processes in terms of nitrogen cycle [14]. It is an exclusive venture by the microbial communities, and one of the factors reflected in the activities of this enzyme are the overall size and activity levels of the microbial communities in the soil. Microbial production of urease is also indirectly regulated by the presence of NH_4^+ and/or NO_3^- ion in the soil sample; their presence will inhibit urease production [15]. However, once in the soil matrix, immobilization or absorption of urease on soil particles and soil organic matter has been shown to stabilize its activities [16] [17]. Finer scale variation in urease activity could therefore be the effect of variable concentrations of the enzyme substrate and products, and also the microbial activities in the soil matrix.

Previous study showed that associated rise in soil pH may increase the soil urease activity and it finally facilitates in hydrolysis of urea which is used as fertilizer in the cultivation field, into NH_3 and CO_2 [18] [19]. Ammonia produced by the action of soil urease activity may escape from the soil because of volatile nature of ammonia [20] [21]. Due to this participation of soil urease regarding Nitrogen mineralization, urease activities in soils have established a lot of concentration because it was first explained by Rotini (1935) [22], an essential process in the regulation of Nitrogen supply to plants after fertilizing with urea. Polacco reported in 1977 [23] that plants mainly secrete soil urease and previous study reported that microorganisms may also secrete urease as both intra-cellular and extra-cellular enzymes [24] [25]. Surprisingly, after secretion from plants or microorganisms, urease being protein in nature can easily be degraded by soil by proteolytic enzymes [12] [26]. This may imply that a considerable fraction of ureolytic activity in the soil may be carried out by extracellular urease, which thus can be stabilized by immobilization on organic and mineral soil colloids. Urease activity in soils can be influenced by many physico-chemical parameters viz. cropping history, organic matter content of the soil, soil depth, soil amendments, concentration of heavy metals, temperatures etc. [27] [28]. Generally, urease activity increases with increasing temperature until a critical temperature is reached when denaturation of the protein present in urease enzyme is initiated and the reaction rate begins to decrease [29]. It is suggested that higher temperatures increase the activity coefficient of this enzyme. The present study explored seasonal, spatial and vertical abundance of soil urease activity along with ammonia content and their correlative interaction relating to Nitrogen mineralization and probable influences of soil parameters on soil urease activity. The hypothesis of the study depicted that impact of soil temperature on urease activity would play a more crucial role than soil salinity in relation to Nitrogen cycle in the Sundarban Mangrove Forest, India.

2. Methods and Materials

2.1. Study Area

The Sundarban Mangrove forest is located geographically in between 21˚31'N and 22˚30'N and longitude 88˚10'E and 89˚51'E along the North East coast of Bay of Bengal, India. This mangrove forest is a part of the estuarine system of the River Ganges, NE coast of Bay of Bengal (**Figure 1**), which covers 9630 km^2. Several numbers of discrete islands constitute Sundarban. The climate in the region is characterized by the southwest monsoon (June-September), northeast monsoon or post-monsoon (October-January), and pre-monsoon (February-May) with 70% - 80% of annual rainfall occurs during the summer monsoon

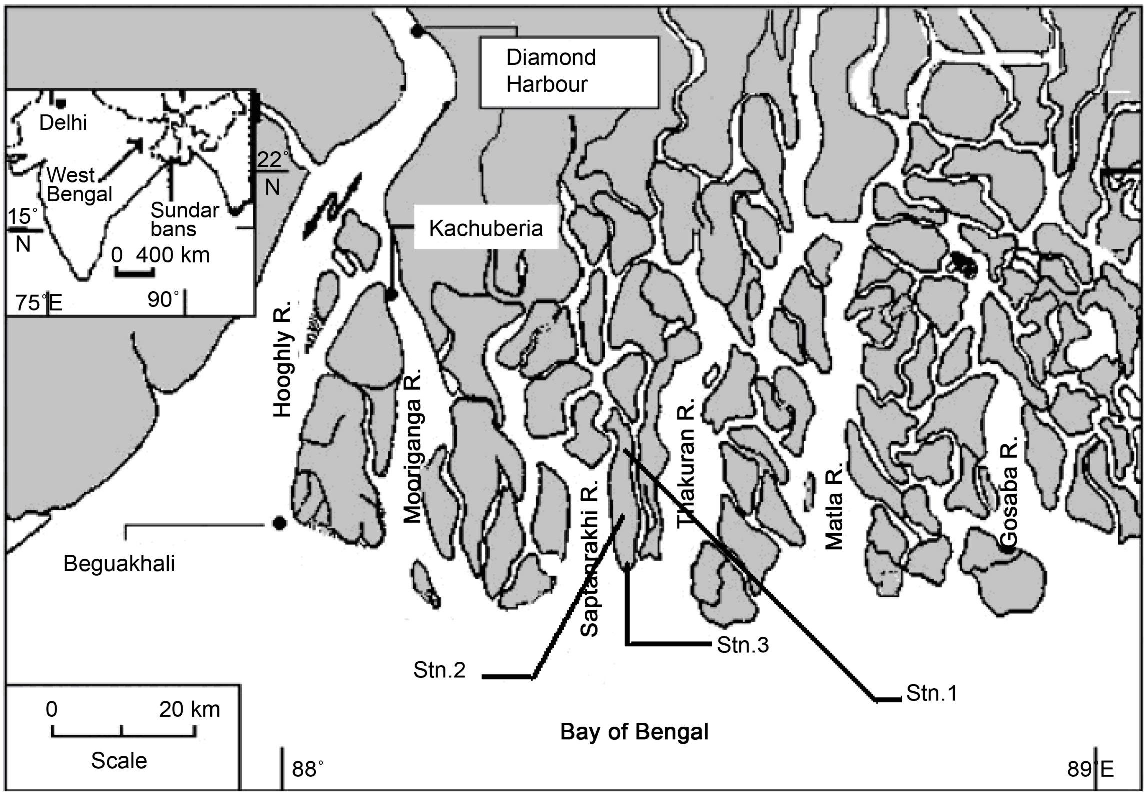


Figure 1. Map showing the study area.

(southwest monsoon). The tide in this estuarine complex is semidiurnal in nature with spring tide ranging between 4.27 m and 4.75 m and neap tide range between 1.83 m and 2.83 m. It is a unique bioclimatic zone in between the land and ocean boundaries of the Bay of Bengal and the largest delta on the globe. The deltaic terrain of Sundarban Biosphere Reserve comprises mainly saline alluvial soil consisting of clay, silt, fine and coarse sand particles.

2.2. Description of the Three Sampling Stations

Sediment cores were collected from three distinct zones namely Deep forest region [in which no tidal action and wave attack occurs as it is furthest from river shore and it contains maximum content of organic carbon and minimum soil salinity and silicate concentration. In this zone plenty of pneumatophores, below ground root and dense vegetation are found (Station 1)], Rooted region [which is situated in between Deep forest region and Un-rooted region. This region contains only pneumatophores but it is devoid of long roots and vegetations. It faces wave attack and tidal action less than that of Un-rooted region (Station 2)], & Un-rooted regions [which is closest to river shore and faces maximum wave attack and tidal action; it contains minimum organic carbon but maximum soil salinity and silicate concentration. This zone is totally devoid of any roots, pneumatophores and vegetations (Station 3)] of Sundarban mangrove ecosystem in every month from six different depths *i.e.* 1) 0 - 10 cm, 2) 10 - 20 cm, 3) 20 -

30 cm, 4) 30 - 40 cm, 5) 40 - 50 cm, & 6) 50 - 60 cm, aseptically using a hand-held stainless steel core sampler (3.2 cm diameter, 100 cm long). Samples were collected into sterilized containers and immediately transferred to the laboratory for analyses.

2.3. Determination of Soil Parameters

2.3.1. Extraction of Soil Samples

Soil sub samples of 30 g were weighed and transferred to 100 ml stopper conical flask and was shaken with exactly 75 ml of 2 mol·L^{-1} potassium chloride (KCl). The mixture was shaken until well mixed and allowed to stand overnight. The samples were filtered into Buchner funnels by using filter papers Whatman no. 42. In the case of turbid filtrates, they were centrifuged by using 3000 cycle/min centrifuge for 5 min. Finally desired volume of the supernatant was collected for the estimation of concentration of ammonia.

2.3.2. Determination of Ammonia in Soil [Phenol-Hypochlorite Method (Grasshoff, 1983)]

Accurately 50 ml of suitably diluted (dilution was done by distilled water) soil extracted solution was taken and concentration of ammonia was determined following standard method [30].

2.3.3. Organic Carbon in Soil Sample: (Walkey and Black, 1934)

Organic carbon in sediment was measured by Walkey-Black method. 10 ml 1N potassium dichromate ($K_2Cr_2O_7$) and 20 ml conc. sulphuric acid solution mixed with silver sulphate (1.25 g Ag_2SO_4 with 100 ml H_2SO_4) were added with 1g dried sediment sample. After allowing it to stand for 30min mixture was diluted with 200 ml distilled water followed by the addition of 85% conc. phosphoric acid (H_3PO_3) and 1 ml of diphenyl amine (0.5 g diphenyl amine in the mixture of 100 ml of conc. H_2SO_4 and 20 ml distilled water) indicator. Mixture was titrated with Mohr salt (393.13 g Mohr salt was dissolved in distilled water, in presence of 50 ml conc. sulphuric acid and the volume was made upto 1 L) until the blue colour was changed to brilliant green. Same procedure was followed for blank without using sediment [31].

2.3.4. Measurement of Soil Temperature, Ph and Eh Value

The pH value was measured in a 1:5 (w/w) soil water suspension using an electric digital pH meter [31] and salinity of a soil saturation extract (ECe) was determined by measuring the electrical conductance of soil extract with the help of a conductivity meter [32]. Soil temperature was measured at different depths using thermocouple probes (±0.1˚C accuracy). Soil redox potential value (Eh) at each sampling depth from three distinct sampling zones was with brightened platinum electrodes which were allowed to equilibrate in situ for 1 hr prior to measurement. Each electrode was checked before use with quinhydrone in pH 4 and 7 buffers (mV reading for quinhydrone is 218 and 40.8, respectively, at 25˚C). The potential of a calomel reference electrode (+244 mV) was added to each value to calculate Eh. Eh values were not corrected for pH, since differences between paired sites were negligible (mean pH = 6.73 ± 0.05 for all sites). A correction for temperature was also not calculated since Eh values change less than 1 mV for every ˚C [33] [34].

2.3.5. Determination of Soil Urease Activity

Moist soil sample of 1.5 g from different sapling zone was incubated with 0.5 ml of 79.9 mM urea (substrate) solution for 2 hr at in situ temperature. The released NH_4^+ from urea by the soil urease was determined spectrophotometrically at 660 nm after treatment of the incubated soil sample with KCl and HCl. The activity of soil urease has been expressed as μg NH_4^+-N g^{-1} dry wt of soil [35] .

2.3.6. Statistical Analysis

All the statistical analyses were done using a MINITAB (version 13.0) statistical package.

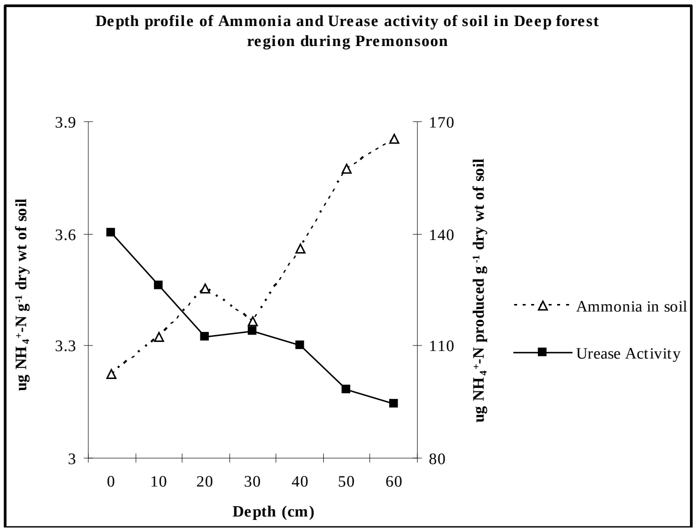
3. Result and Discussion

A depth wise seasonal study was done for soil urease enzyme activity along with ammonia content from 1) Deep forest region, 2) Rooted region and 3) Un-rooted region.

3.1. Deep Forest Region

Maximum soil urease activity (178.67 μg NH_4^+-N produced g^{-1} dry wt of soil) was found from the surface soil of Deep forest region during Post monsoon and minimum urease activity (69.25 μg NH_4^+-N produced g^{-1} dry wt of soil) was found from the 60 cm of depth during monsoon. In three seasons soil urease activity decreased with increase in depth in the Deep forest region. Previous research study reported that urease activity might decrease with increase in depth [26] [36]. A reverse profile for ammonia content of soil was found (**Figures 2(a)-(c)**). Maximum ammonia (6.19 μg NH_4^+-N g^{-1} dry wt of soil) content was found from the soil of 60 cm of depth during post monsoon whereas minimum value (1.43 μg NH_4^+-N g^{-1} dry wt of soil) was found from the surface soil during monsoon. It may be predicted that decreasing organic carbon with increase in depth causes lowering of microbial load which ultimately reflects on lowering of soil urease activity with increasing depth [25]. More anoxic condition in the deeper soil may inhibit the oxidation of ammonia for which in the Deep forest region the content of ammonia in soil was found to increase with increasing depth [37].

A negative correlation was found for the content of ammonia in soil and soil urease activity [The regression equation is ammonia in soil = −1.64 + 0.0402 Urease Activity (R-Sq = 62.9%, $P < 0.001$, n = 41)]. A decreasing pattern of salinity, organic carbon and temperature was found with increase in depth. Maximum of soil organic Carbon (1.37%) was found during post monsoon from the surface soil and minimum organic Carbon (0.59%) was found during monsoon



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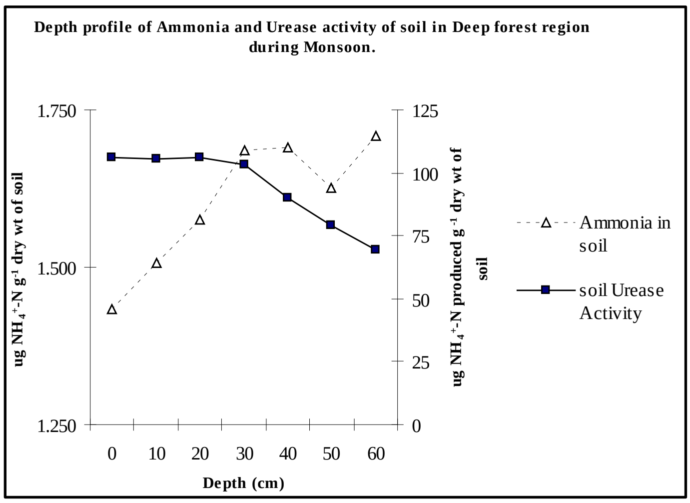

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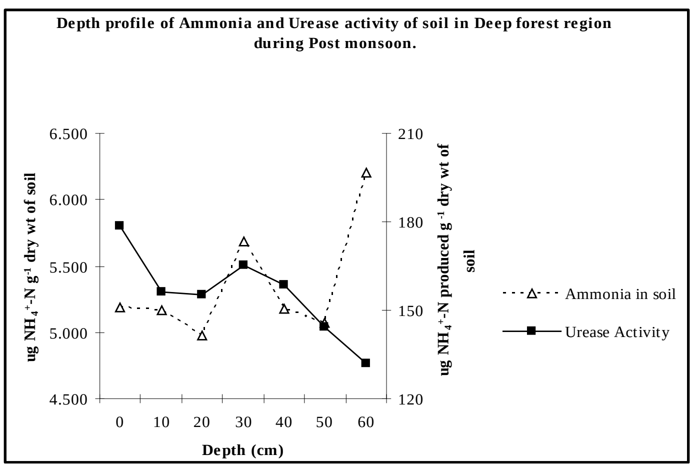


(c)

Figure 2. Depth profile of the content of Ammonia in soil and soil Urease activity in the Deep forest region during Premonsoon (a), Monsoon (b) and Post monsoon (c).

season from the soil of 60 cm of depth [38]. The distinct stratification of soil urease, ammonia content, organic Carbon was found in the deep forest region which may be attributed to more vertical stability of the soil in that region [39]. Maximum salinity (20.06 psu) was found from the 60 cm of depth during postmonsoon and minimum (15.37 psu) was found during monsoon season from the surface soil. Maximum soil temperature (24.70˚C) was found during monsoon from the 10 cm of depth whereas minimum soil temperature (12.93˚C) was found during post-monsoon from the 30 cm of depth. Maximum of soil pH (8.42) was found during post-monsoon from the surface soil and minimum pH (7.94) was found during pre-monsoon from the surface soil of deep forest region (**Table 1**).

3.2. Rooted Region

In rooted region similar pattern of vertical distribution of ammonia content and soil urease activity was found in three seasons. Maximum soil urease activity (156.24 μg NH_4^+-N produced g^{-1} dry wt of soil) was found from the surface soil

Table 1. Seasonal and vertical fluctuation of pH, temp, Salinity and organic carbon in the soil of deep forest region.

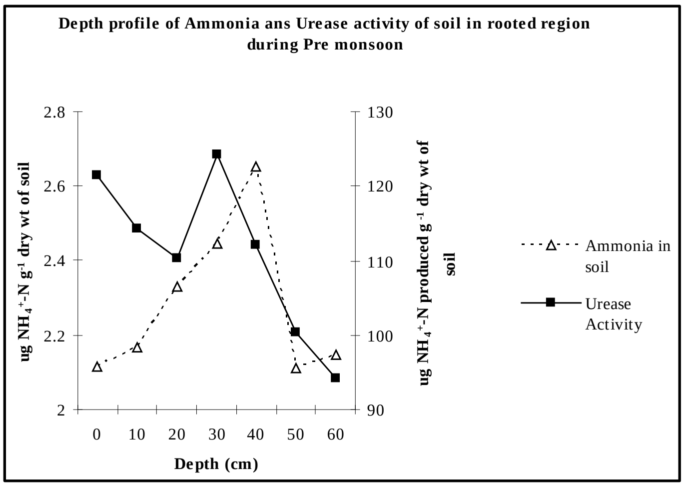
Season	Depth (cm)	Avg. pH	Avg. Temp (˚C)	Avg. Salinity (PSU)	Org.C (%)
Premonsoon	0	7.94	17.83	15.62	1.03
	10	8.394	17.83	15.95	0.97
	20	8.273	17.82	16.15	0.92
	30	8.23	17.81	16.12	0.82
	40	8.251	17.82	16.32	0.78
	50	8.214	17.8	16.5	0.75
	60	8.194	17.82	16.85	0.7
Monsoon	0	8.218	24.68	15.37	0.87
	10	8.17	24.7	15.7	0.82
	20	8.185	25.17	15.85	0.8
	30	8.18	24.69	15.92	0.83
	40	8.136	24.59	16	0.7
	50	8.156	23.82	16.05	0.67
	60	8.119	23.73	16.37	0.59
Post monsoon	0	8.423	12.943	18.76	1.37
	10	8.365	12.945	19.29	1.26
	20	8.338	12.938	19.32	1.25
	30	8.32	12.93	19.39	1.07
	40	8.24	12.9	19.68	0.97
	50	8.24	12.923	19.93	0.92
	60	8.185	13.123	20.06	0.93

of rooted region during Post monsoon and minimum urease activity (49.45 μg NH_4^+-N produced g^{-1} dry wt of soil) was found from the 60 cm of depth during monsoon. Ammonia content in soil was found to increase with increase in depth which is just mirror image or vertical distribution of soil urease activity. Maximum ammonia (4.87 μg NH_4^+-N g^{-1} dry wt of soil) content was found from the soil of 60 cm of depth during post monsoon whereas minimum value (0.98 μg NH_4^+-N g^{-1} dry wt of soil) was found from the soil of 20 cm of depth during monsoon (**Figures 3(a)-(c)**). In rooted region maximum of soil organic Carbon (1.13%) was found from the surface soil during post-monsoon and minimum organic Carbon (0.52%) was found during pre-monsoon from the 60 cm of depth. Maximum salinity (20.79 psu) was found from the 50 cm of depth during post-monsoon and minimum (15.63 psu) was found during monsoon season from the surface soil. Maximum soil temperature (25.27˚C) was found during monsoon from the 20 cm of depth whereas minimum soil temperature (12.71˚C) was found during post-monsoon from the 50 cm of depth. Maximum of soil pH (8.29) was found during post-monsoon from the surface soil and minimum pH (7.98) was found during monsoon from the 40 cm of depth (**Table 2**).

3.3. Un-Rooted Region

In Un-rooted region no distinct vertical stratification was found for both ammonia content in soil and soil urease activity. Maximum soil urease activity (126.45 μg NH_4^+-N produced g^{-1} dry wt of soil) was found from the soil of 50 cm of depth during Post monsoon and minimum urease activity (55.33 μg NH_4^+-N produced g^{-1} dry wt of soil) was found from the 60 cm of depth during Premonsoon. Maximum ammonia (3.35 μg NH_4^+-N g^{-1} dry wt of soil) content was found from the soil of 40 cm of depth during post monsoon whereas minimum value (0.63 μg NH_4^+-N g^{-1} dry wt of soil) was found from the surface soil during monsoon (**Figures 4(a)-(c)**). In un-rooted region maximum of soil organic Carbon (1.00%) was found from the surface soil during post-monsoon and minimum organic Carbon (0.42%) was found during monsoon from the 20 cm of depth. Maximum salinity (21.58 psu) was found from the 60 cm of depth during post-monsoon and minimum (16 psu) was found during monsoon season from the 20 cm of depth. Maximum soil temperature (25.22˚C) was found during monsoon from the surface soil whereas minimum soil temperature (12.88˚C) was found during post-monsoon from the 50 cm of depth. Maximum of soil pH (8.33) was found during post-monsoon from the 40 cm of depth and minimum pH (7.64) was found during monsoon from the 60 cm of depth (**Table 3**). No distinct stratification of soil urease, ammonia content, organic Carbon was found in the un-rooted region which may be attributed to less vertical stability of the soil in that region due to extreme wave attack [40].

Stepwise regression analysis of data (**Table 4**) showed that soil urease activity could explain only 0.1% of the variation of soil salinity compared to 52.6%, 9.3%, 12.8% and 1.9% by pH, T (Temperature in ˚C), Org.C (%) and soil ammonia



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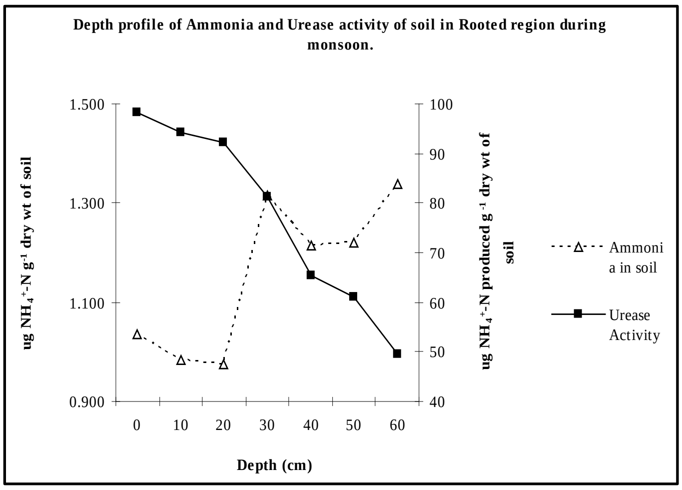

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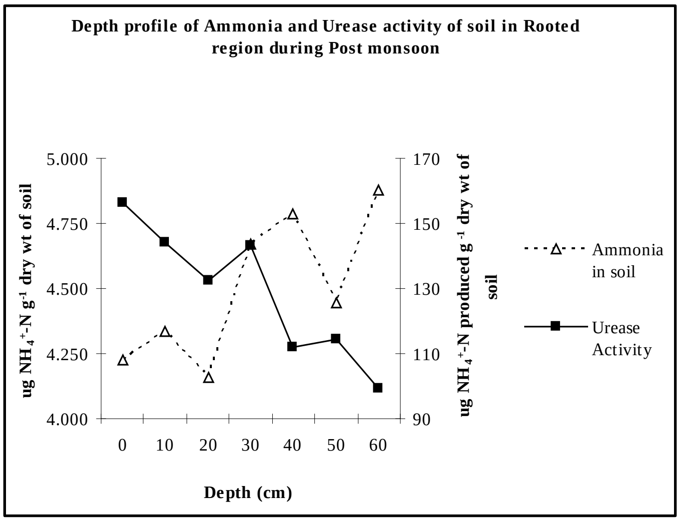

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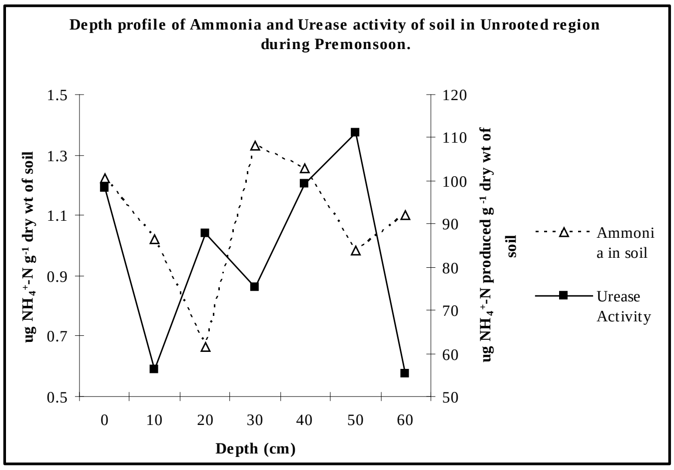
Figure 3. Depth profile of the content of Ammonia in soil and soil Urease activity in the Rooted region during Premonsoon (a), Monsoon (b) and Post monsoon (c).



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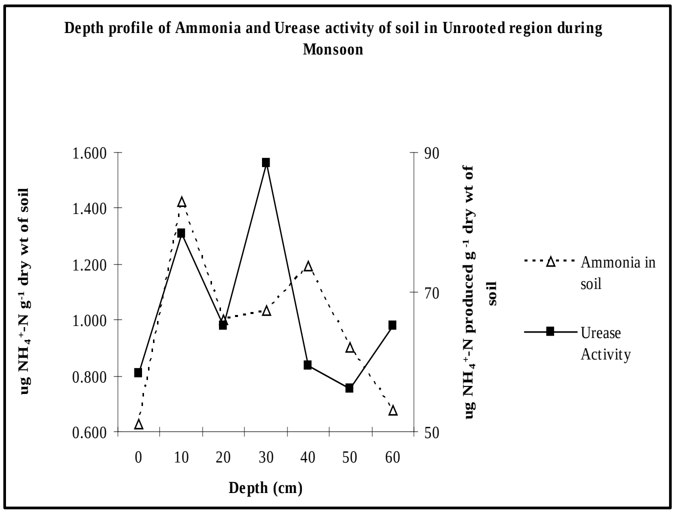

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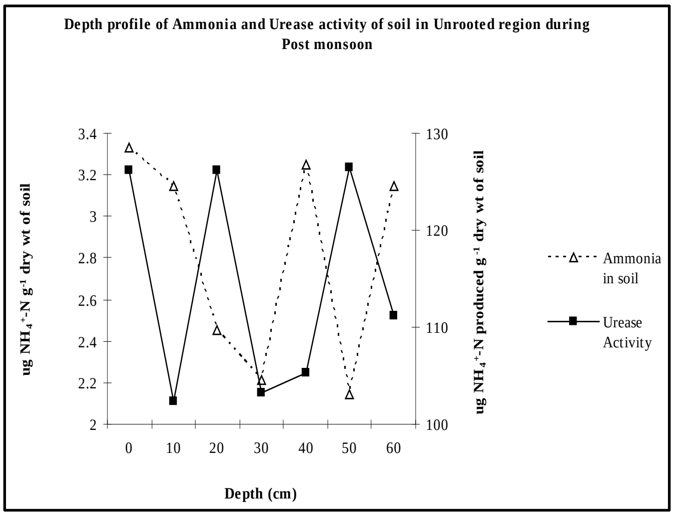

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Figure 4. Depth profile of the content of Ammonia in soil and soil Urease activity in the Unrooted region during Premonsoon (a), Monsoon (b) and Post monsoon (c).

Table 2. Seasonal and vertical fluctuation of pH, temp, salinity and organic carbon in the soil of rooted region.

Season	Depth (cm)	Avg. pH	Avg. Temp (˚C)	Avg. Salinity (PSU)	Org.C (%)
	0	8.207	18.27	16.875	0.64
	10	8.193	18.26	16.85	0.56
	20	8.164	18.25	16.775	0.6
Pre monsoon	30	8.219	18.25	16.74	0.63
	40	8.196	18.24	17.275	0.52
	50	8.006	18.24	17.55	0.53
	60	8.118	18.23	17.2	0.52
	0	8.119	24.71	15.63	0.83
	10	8.025	24.73	15.9	0.77
	20	8.092	25.27	16.15	0.71
Monsoon	30	8.01	24.81	15.87	0.72
	40	7.986	24.75	16.22	0.64
	50	8.026	23.93	16.45	0.63
	60	8.024	23.89	16.87	0.58
	0	8.29	12.73	20.13	1.13
	10	8.25	12.72	20.34	0.94
	20	8.25	12.71	20.03	0.93
Post monsoon	30	8.23	12.71	19.76	0.79
	40	8.15	12.7	20.46	0.79
	50	8.158	12.7	20.79	0.83
	60	8.165	12.9	20.72	0.86

content (μg NH_4^+-N produced g^{-1} dry wt of soil) respectively. Previous study showed that soil temperature had significant control over soil urease activity [41]. This result signified that change in soil salinity of the Sundarban mangrove ecosystem did not affect the microbial activity, compared to soil temperature and pH, relating to soil urease activity, which intern assigns the salt tolerance ability of the soil urease activity of that ecosystem.

4. Conclusion

The present study revealed that Deep forest region contained more ammonia, organic carbon, soil urease activity than Rooted and Un-rooted region. Soil urease activity was sensitive to soil temperature ant soil pH value, whereas salinity did not affect too much on urease activity due to salt tolerance ability. Soil urease activity decreased with increase in depth mainly in undisturbed Deep forest

Table 3. Seasonal and vertical fluctuation of pH, temp, salinity and organic carbon in the soil of unrooted region.

Season	Depth (cm)	Avg. pH	Avg. Temp (˚C)	Avg. Salinity (PSU)	Org.C (%)
Pre monsoon	0	8.169	18.41	17.13	0.55
	10	8.091	18.35	16.85	0.51
	20	8.161	18.43	17	0.49
	30	8.079	18.36	16.27	0.59
	40	8.213	18.31	17.27	0.54
	50	8.107	18.35	17.47	0.58
	60	8.038	18.42	16.4	0.65
Monsoon	0	7.954	25.21	16.5	0.5
	10	7.868	25.19	17.1	0.45
	20	7.944	25.03	16	0.42
	30	7.824	24.61	17.57	0.49
	40	7.854	24.74	16.85	0.48
	50	7.764	24.75	17.17	0.47
	60	7.636	24.32	18.42	0.49
Post monsoon	0	8.288	12.92	20.75	1
	10	8.245	12.95	21.54	0.92
	20	8.323	12.91	20.78	0.93
	30	8.26	12.9	20.34	0.79
	40	8.33	12.89	21.48	0.9
	50	8.305	12.88	20.51	0.83
	60	8.213	12.91	21.58	0.65

Table 4. Multiple regression analysis with a stepwise variable selection (n = 63)*.

Predictor	R^2	F	P
Soil pH	52.6	67.57	<0.001
Soil Temp	61.9	48.77	<0.001
Soil Salinity	62	32.1	0.703
Org. C	74.8	43.1	<0.001
Ammonia content	76.7	37.44	<0.001

*The dependent variable, soil Urease Activity (μg NH_4^+-N produced g^{-1} dry wt of soil) is not shown. Urease Activity = −236 + 37.4 pH − 0.900 T − 0.085 S + 53.7 Org. C + 5.46 Ammonia. The independent variables are pH, T (Temperature in ˚C), salinity (S, PSU), Org. C (%) and Ammonia (μg·g^{-1} dry wt of soil).

region. No distinct stratification was found for soil urease activity and ammonia content because of wave attack and other disturbances in that zone.

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