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Microbial Biomass Nitrogen as an Index of N Availability in Acidic Soils of North East India

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ABSTRACT

A reliable estimate of soil's nitrogen (N) supplying capacity is essentialto improve N fertilizer efficiency and crop productivity.In the study reported here, we evaluated the utility of soil microbial biomass N (SMB-N) *vis-à-vis*alkaline $KMnO_4$ extractable-Nas an index of N availability in acidic soils of northeast India. These indices were evaluated based on their correlation with available N obtained through two standard incubation methods *viz*., aerobic incubation (AI-N) and anaerobic incubation (ANI-N), and also with dry matter yield, N concentration and N uptake (PNU) in maize (*Zea mays* L.). SMB-N correlated significantly better with AI-N and ANI-N ($r = 0.836^{**}$ and 0.811^{**} respectively) compared to alkaline $KMnO_4$-N($r = 0.394^{**}$ and 0.548^{**} respectively). Correlations of plant parameters were also stronger with SMB-N (r=0.707** for PNU) than with alkaline $KMnO_4$-N (r= 0.625**).Based on the significantly stronger relationship of SMB-N with the standard biological indices and the plant responses, we envisage SMB-N as a reliable index of N availability in acidic soils of northeast India.

Keywords: Biological incubation, Chemical extraction, Maize, N supplying capacity

INTRODUCTION

Nitrogen (N) is often the most limiting nutrient for crop production in majority of the world soils, including those in India. To bridge the gap between plant N demand and available N in soil, supply of the nutrient from external sources is imperative. An accurate estimate of potentially available N in soil is therefore essential to ensure optimum crop yield and quality and also to minimize nutrient loss to environment that may result from overuse of fertilizers. Among the numerous methods proposed for assaying the N-supplying capacity of soils, biological methods (aerobic and anaerobic incubation)are considered to be most reliable. The precision of biological methods gets reflected most often in higher correlations of N availability estimates obtained through them with yield and N uptake by plants. However, owing to tedious and time consuming nature of these biological methods, they are not considered suitable for routine estimation of plant available N in soil. In pursuit of developing a rapid, yet reliable, index of soil N availability, many chemical indices have been proposed over time, but no single method has performed consistently enough to receive broad acceptance across a wide range of soils. In India, alkaline permanganate extraction procedure, as proposed by Subbiah and Asija (1956) and modified by Stanford (1978), has been the most preferred method of estimating available N in soils, including the acidic soils of northeast India. However, effectiveness of this method as a reliable predictor of N availability in the soils of this region has not been tested adequately. Furthermore,there are reports that indicate the poor relationship between soil N availability obtained through this method and plant responses. This necessitates the evaluation of alkaline $KMnO_4$ extractable-N as a predictor of N availability in acidic soils of northeast India.

Soil microbial biomass nitrogen (SMBN) has been successfully used by many researchers as a reliable predictor of N availability (Hu and Cao 2007; Sharifi et al. 2007). Carter and Macleod (1987) found SMBN to be closely related to potentially mineralizable N in soil ($R^2 = 0.94$). Jin et al. (2007) also reported a good correlation of SMBN ($r = 0.665$) with N mineralization potential

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of soil and hence suggested its use as a biological index of soil N availability. The relative ease of determining SMBN in comparison to the incubation-based biological methods also makes it a better proposition for assessing soil N availability. However, there is serious dearth of information regarding relationship between SMBN and other established biological indices viz., aerobic incubation (AI) and anaerobic incubation (ANI). The existing knowledge on correlation of SMBN with plant parameters (viz., dry matter yield, plant N content and N uptake (PNU) is also inadequate. In this context, the present study was undertaken to evaluate the performance of soil microbial biomass N (SMB-N) vis-à-vis alkaline $KMnO_4$ extractable-N as an index of N availability in acidic soils of northeast India. The evaluation was made based on their strength of correlation with the available N obtained through two standard incubation methods viz., aerobic incubation (AI-N) and anaerobic incubation (ANI-N), and also with dry matter yield, N concentration and N uptake (PNU) in maize (*Zea mays* L.).

MATERIALS AND METHODS

Experimental soils

The soils used in the study were collected from 20 representative sites in the state of Meghalaya, northeast India. Using the previously available information, these sampling sites were selected to accommodate a wide variation in soil properties, including total N, available N and organic C. The bulk soils collected from 0-20 cm depth were shipped to laboratory, air-dried and passed through 2-mm sieve (0.5 mm for organic C) for further analysis. Samples were analysed for initial physico-chemical properties as follows: soil pH was measured with a glass electrode in a 1:2.5 (w/v) soil/water suspension; organic C by Walkey-Black method (1934); total N by kjeldahl method (Bremner 1996); available N by alkaline $KMnO_4$ distillation method (Subbiah and Asija 1956); available P by Bray-II method (Bray and Kurtz 1945). Exchangeable K and Na was determined using ammonium acetate extraction followed by emission spectrometry (Jackson 1973). Exchangeable Ca and Mg were determined by versene titration (Baruah and Barthakur 1999); exchangeable-Al and acidity by neutral KCl extraction method (Page et al. 1982) and cation exchange capacity (CEC) by sodium saturation method (Jackson 1973). Percent base saturation (PBS) was estimated as the proportion (%) of CEC contributed by exchangeable bases (Na, K, Ca, and Mg). Percent sand, silt and clay were determined by international pipette method (Piper1966). Some pertinent physico-chemical properties of the experimental soils are shown in table 1.

Pot experiment

To work out the correlation between N availability indices and the plant responses (dry matter yield, N concentration, and N uptake), a pot experiment was conducted with maize (*Zea mays* L.) as a test crop. Maize seeds (Cv. RCM 1-1) were sown in plastic pots containing 7.5 kg of experimental soils with four replications. The plant was harvested at the initiation of tasseling stage, and the dry matter weights were recorded after oven drying at 70ºC for 48 hours. N concentration in maize tops was estimated using micro kjeldahl procedure (Jackson 1973) and the N uptake was subsequently worked out.

Methods of assessing potentially available soil-N (N availability indices)

The performance of SMBN as a predictor of potentially available soil N was assessed against two biological incubation methods (as reference) and one of the most commonly used chemical methods as briefed below. Soil microbial biomass N (SMBN) was extracted using 0.5*M* K_2SO_4 following chloroform fumigation extraction procedures (Anderson and Ingram 1993).

Aerobic incubation

The method involves estimation of the (exchangeable ammonium + nitrate + nitrite)-N produced when 10 g of soil mixed with 30 g of quartz sand are treated with 6 ml of water and incubated at 30ºC for 14 days under conditions which ensure adequate aeration without loss of water (Keeney and Bremner 1966). Briefly, a mixture of 10 g soil with 30 g quartz sand was distributed evenly over the bottom of a 250-ml bottle containing 6 ml of distilled water to bring the moisture content to field capacity. The necks of the bottles were fitted with rubber stoppers having a central hole, sealed tightly with an aeration

Table 1: Physico-chemical properties of the soils used in the study

Sample ID	Sampling site	pH (1:2.5)	Sand (%)	Clay (%)	CEC [Cmol (P^+) kg^{-1}]	Base Saturation (%)	SOC (g kg^{-1})	Total N (mg kg^{-1})	Avail. N (mg kg^{-1})	Avail. P_2O_5 (mg kg^{-1})	Avail. K_2O (mg kg^{-1})
S_1	Nongpoh	4.90	65.44	25.61	12.50	21.28	7.1	1296.1	132.2	14.5	108.9
S_2	Sokhwai	4.50	60.96	29.71	14.80	23.91	17.1	1516.5	249.8	11.3	145.8
S_3	Jirang	4.54	62.77	31.89	14.62	27.91	17.2	1876.8	208.1	8.6	113.6
S_4	Aphrewmer	4.96	68.53	21.46	11.00	23.50	10.2	1488.2	138.8	9.5	121.2
S_5	Nongkrah	4.64	56.11	34.56	14.50	18.97	8.3	1425.2	166.5	10.3	124.8
S_6	Mawphru	4.89	66.34	28.09	13.50	19.70	11.7	1606.1	222.0	13.6	134.3
S_7	Umsning	4.31	51.53	39.04	15.90	18.30	16.5	1421.1	235.2	6.6	151.6
S_8	Mawksiew	5.32	62.29	31.04	14.40	69.22	13.5	1219.2	214.5	10.0	136.4
S_9	Killing	4.60	66.29	28.37	10.63	38.10	15.9	1704.5	203.7	15.3	137.9
S_{10}	Raitong)	4.39	49.01	37.65	19.20	13.02	18.2	1281.5	112.0	7.3	100.3
S_{11}	Umiam	4.27	56.96	31.04	14.50	22.89	17.6	1538.2	102.3	6.0	102.8
S_{12}	Nongpoh	4.69	62.77	25.71	18.30	22.13	23.2	1705.3	251.8	17.0	152.2
S_{13}	Umden	4.99	55.63	32.37	14.20	32.61	19.3	2014.0	223.4	14.9	158.5
S_{14}	Umrit	4.79	58.35	32.37	14.90	13.36	16.4	1333.2	184.6	14.9	138.1
S_{15}	Mawhati	4.64	52.96	35.04	10.80	19.54	9.4	1188.5	95.8	8.8	84.3
S_{16}	Klew	4.69	74.08	21.92	9.90	56.56	13.2	1615.6	172.0	5.9	96.7
S_{17}	Myrdon	4.70	63.68	28.37	10.10	19.91	12.5	1316.8	138.0	6.8	118.6
S_{18}	Umsning	5.05	51.63	34.04	10.60	43.39	12.2	1456.9	176.4	14.4	130.0
S_{19}	Mawpun	4.56	50.29	33.71	13.10	45.27	8.6	1296.1	138.9	14.1	118.4
S_{20}	Mynsain	4.79	58.29	31.04	11.10	26.31	7.6	1108.8	120.3	12.8	94.1

device, and incubated at 30 ±1°C for 14 days. Thereafter, 100 ml of 2M KCl was added to each bottle. The bottles were then fitted with solid rubber stoppers and shaken for one hour in a mechanical shaker. Thereafter, bottles were allowed to stand until the mixture settled and the supernatant liquid was clear. Twenty ml aliquot of the supernatant was added to a 100-ml distillation flask. The amount of (NH_4+NO_3+NO_2)-N produced from the soil-sand mixture during the incubation period was determined from the NH_4-N liberated by the steam distillation of this aliquot with 0.2g MgO and 0.2g Devarda alloy for 3.3 minutes. The mineralizable-N was calculated as the difference between amount of NH_4-N liberated after and before incubation.

Anaerobic incubation

The method involves incubation of a soil sample under waterlogged conditions in an enclosed test tube with minimum possible head space (Keeney 1982; modified from Waring and Bremner 1964). Briefly, 12.5 ml of distilled water was placed in a test tube (16X150-mm) followed by addition of 5g oven dry equivalent of soil. The test tubes were stoppered and incubated at 40±1°C for 7 days. Thereafter, tubes were removed from the incubator, shaken briefly to mix the content, and the mixture was quantitatively transferred to a 150-ml distillation flask by washing with 15 ml of 4 M KCl solution. About 0.2 g of MgO was added to the mixture and steam distilled for 4 minutes to estimate the amount of NH_4-N liberated. The initial amounts of NH_4-N present in soil were determined by steam distillation of another sub-sample before incubation. The mineralizable N was calculated from the difference between the results of these two analyses.

Alkaline permanganate extraction

The method described by Stanford (1978) was followed for the extraction. Briefly, 1.0 g of air-dried soil was placed in a 100-ml distillation flask. Ten ml of 0.25 M NaOH containing 0.1g of $KMnO_4$ was added to the flask and the contents steam distilled for 4 minutes. The liberated NH_4-N was collected in a 50-ml Erlenmeyer flask containing 5 ml of boric acid-indicator solution. The amount of NH_4-N was determined by titration with standard 0.005 N H_2SO_4. Another 1.0 g of soil sample was treated with 10 ml of 0.25 M NaOH only, steam distilled for 4 minutes and the liberated NH_4-N trapped and estimated as mentioned above. The NH_4-N produced by alkaline $KMnO_4$ oxidation was calculated as the difference between results of the two analyses.

Statistical analysis

Data were statistically analyzed using the SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA). Pearson's correlation coefficient was used to compare the correlation between chemical and biological indices and the plant parameters; significance of these tests was considered at 0.05 and 0.01 probability levels.

RESULTS AND DISCUSSION

Characteristics of the soils and N-availability indices

In this study, SMBN as an index of soil N availability was evaluated against the biological incubation (aerobic and anaerobic) methods, which are often used as the reference methods for predicting N-supplying capacity of soils, and also against one of the most commonly used chemical index of soil N availability (alkaline $KMnO_4$-N). The indices were further correlated with plant responses (dry matter yield, percent N and plant N uptake) which provide a more realistic assessment of soil's capacity to supply N to growing crops (Dalal and Mayer 1990). In general, the soils used in the study showed large variability in N-supplying capacity as indicated by the differential response of growth and N uptake by plants grown thereon (Table 2). Since dry matter yield of plant is influenced by too many factors other than available N, plant N uptake (PNU) is taken as a more realistic indicator of N availability in soils (Sahrawat 1983; Hussain et al. 1984; Li et al. 2011). Thus, a large variability in PNU ranging from 598 mg pot^{-1} (S_{10}) to 1026 mg pot^{-1} (S_{12}) is indicative of the varying N-supplying capacities of soils under study. Organic C content of soils ranged between 7.1 to 23.2 g kg^{-1}, total N between 1109 to 2014 mg kg^{-1}, clay content between 21.92 to 39.04 %, CEC between 9.9 to 19.2 Cmol (P^+) kg^{-1}, base saturation between 13 to 57 %, and so varied the other soil properties as well (Table 1).

Of the two biological methods of determining soil N-availability, anaerobic incubation (ANI), on average, yielded more value than the aerobic incubation (AI) (20.72 and 49.94 mg N kg^{-1} soil,

Table 2: Dry matter yield and N uptake by plant, and various indices of N availability in soil as estimated by biological incubations and chemical extraction methods

	Plant response			Indices of available N in soil (mg kg^{-1})			
Sample ID	Dry matter yield (g pot^{-1})	N content (%)	N uptake (mg pot^{-1})	AI-N	ANI-N	SMB-N	Alk. $KMnO_4$-N
S_1	72.5	0.87	631	9.74	34.56	11.18	107.4
S_2	80.6	0.97	782	15.51	52.65	9.95	185.4
S_3	75.6	0.91	688	12.98	35.11	15.77	226.0
S_4	73.3	0.88	645	10.68	38.80	13.97	121.1
S_5	72.0	0.86	619	10.29	35.18	11.66	144.6
S_6	76.8	0.92	707	13.77	41.23	14.78	212.6
S_7	83.3	1.00	833	36.95	79.68	23.17	182.1
S_8	81.1	0.98	795	24.42	61.18	19.76	192.2
S_9	82.6	0.99	818	24.83	65.54	12.22	200.3
S_{10}	70.4	0.85	598	9.23	26.18	9.31	89.4
S_{11}	71.2	0.86	612	9.42	33.49	13.35	77.8
S_{12}	92.4	1.11	1026	78.30	107.66	32.92	149.3
S_{13}	88.2	1.06	935	48.71	87.18	26.12	229.8
S_{14}	80.7	0.97	783	31.73	74.21	22.63	201.0
S_{15}	70.1	0.84	589	9.39	28.59	12.38	72.4
S_{16}	78.9	0.95	750	14.66	39.44	13.29	154.6
S_{17}	75.5	0.91	687	13.37	38.12	15.61	115.4
S_{18}	80.4	0.97	780	15.23	48.75	15.58	161.4
S_{19}	74.7	0.90	672	12.69	36.18	12.79	97.7
S_{20}	74.6	0.90	671	12.50	35.07	12.91	116.9
Mean	77.7	0.93	723	20.72	49.94		151.9

Each datum is the average of four replications; AI-N – available-N through aerobic incubation; ANI-N – available-N through anaerobic incubation; SMB-N: soil microbial biomass N

respectively). This is in conformity with the results obtained by Elkarim and Usta (2001). Greater amounts of mineralized N obtained under ANI could be attributed to the fact that the losses of ammonia which may occur under AI were avoided in the enclosed system of ANI. Also, higher temperature used in ANI (40ºC) than in AI (30ºC) might have resulted in higher values of mineralised N (Bremner 1965; Keeney 1982). Out of all the indices under study, alkaline $KMnO_4$, on average, extracted the highest amount of N (151.9 mg kg^{-1}). Similar extracting ability of N by alkaline $KMnO_4$ was also reported by Nayyar et al. (2006) and Li et al. (2011). Soil microbial biomass nitrogen (SMBN) yielded the lowest values (15.97 mg N kg^{-1} soil).

Relationships between soil N-availability indices and plant response

Correlations between all the pairs of soil N availability indices were found significant (Table 3). The highest coefficient of correlation (r = 0.946**, p<0.01) was obtained between AI-N and ANI-N the two standard indices of soil N-availability. The next stronger correlation observed was that of SMBN with AI and ANI (r = 0.836** and 0.811**, respectively). Interestingly, alkaline $KMnO_4$-N showed least correlations with AI-N and ANI-N (r = 0.394** and 0.548**, respectively).

Table 3: Correlation coefficients (*r*) between indices of soil N availability

	AI-N	ANI-N	SMB-N	Alk. $KMnO_4$-N
AI-N	1			
ANI-N	.946**	1		
SMB-N	.836**	.811**	1	
Alk. $KMnO_4$-N	.394**	.548**	.390**	1

*P < 0.05; **P < 0.01. AI-N: available-N through aerobic incubation;
ANI-N:available-N through anaerobic incubation; SMB-N: soil microbial biomass N

Correlations of the N-availability indices with plant parameters are shown in table 4. Highest correlations with plant N uptake (PNU) were shown by the biological indices, of which, ANI-N showed stronger correlations (r = 0.920**) than the AI-N (r = 0.876**). This was followed by SMBN, which recorded a correlation coefficient of 0.707** with PNU. Of all the indices of N availability under study, alkaline $KMnO_4$-N registered the lowest correlation (r = 0.625**) with PNU. Correlations of the other two plant parameters (dry matter yield and N concentration) with various N-availability indices showed similar trend and strength as with PNU.

Table 4: Correlation coefficients (*r*) between plant parameters and the indices of soil N availability

	AI-N	ANI-N	SMB-N	Alk. $KMnO_4$-N
Dry matter yield	.885**	.925**	0.711**	.633**
N Concentration	.876**	.928**	0.723**	.631**
N uptake	.876**	.920**	0.707**	.625**

*P < 0.05; **P < 0.01. AI-N: available-N through aerobic incubation;
ANI-N: available-N through anaerobic incubation; SMB-N: soil microbial biomass N

As anticipated, the biological incubation methods, being the most reliable predictors of soil N availability registered the strongest correlations among themselves and also with plant parameters. The better reliability of these methods as compared to the chemical indices was further confirmed by the higher levels of their correlation with plant parameters including PNU, as frequently reported in literature (Sahrawat 1983; Hussain et al. 1984). The strong correlation of SMBN with AI-N and ANI-N and also with the plant parameters suggests the worth of the procedure in describing the potential N supplying capacity of the soil. Similar findings regarding SMBN as a predictor of soil's potential N supplying capacity was also reported by Carter and Macleoid (1987). The ability of SMBN in predicting soil's N supplying capacity in this study perhaps implies the importance of microbially transformed N as the plant available N pool. In situations like Meghalaya, where the loss of N through processes like leaching etc. is rampant, the quantity of N microbially transformed into plant available form at a particular point of time bears utmost importance. This might be the reason behind such reliability of SMBN in predicting soil N availability as observed in this study, although many other workers suggested microbial activity rather than size of the microbial biomass as a better indicator of soil N availability (Puri and Ashman 1998).

Compared to SMBN, alkaline $KMnO_4$ extraction, the most commonly used method for estimating soil N availability in India, remained a poor performer despite yielding significant correlations with the biological incubation procedures and plant parameters. Most of the studies (Subbiah and Asija 1956; Nayyar et al. 2006; Maiti and Das 2007) establishing the applicability of alkaline $KMnO_4$ extraction method for predicting soil N-availability in India were carried out in soils with neutral to alkaline reaction leaving a vast scope for such studies in acidic soils of the country.Many studies have reported that alkaline $KMnO_4$ method does not provide satisfactory results (Bordoloi et al 2012). In a study by Gianello and Bremner (1986), alkaline permanganate method had the poorest precision of the 12 chemical indices used in assessing N availability in Brazilian soils. Further consolidating the results of the present investigation, Elkarim and Usta (2001) reported the poorest correlation of biological incubation methods with alkaline $KMnO_4$-N out of six chemical indices of N availability tested in Central Anatolian soils.

CONCLUSIONS

In comparison with alkaline $KMnO_4$-N, soil microbial biomass N (SMB-N) correlated consistently better with AI-N and ANI-N as well as with plant responses. Thus, based on results of the present study, we envisage SMB-N as a reliable index of N availability in acidic soils of northeast India.

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