Evaluation of nitrogen availability indices and their relationship with plant response on acidic soils of India

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ABSTRACT

Plant's nitrogen (N) requirement that is not fulfilled by available N in soil has to be supplied externally through chemical fertilizers. A reliable estimate of soil N-supplying capacity (NSC) is therefore essential for efficient fertilizer use. In this study involving a pot experiment with twenty acidic soils varying widely in properties, we evaluated six chemical indices of soil N-availability viz. organic carbon (C_{org}), total N (N_{tot}), acid and alkaline-$KMnO_4$ extractable-N, hot KCl extractable-N (KCl-N) and phosphate-borate buffer extractable-N (PBB-N), based on their strength of correlation with available-N values obtained through aerobic incubation (AI-N) and anaerobic incubation (ANI-N), and also with the dry matter yield (DMY), N percentage and plant (maize) N uptake (PNU). In general, the soils showed large variability in NSC as indicated by variability in PNU which ranged from 598 to 1026 mg/pot. Correlations of the N-availability indices with AI-N and ANI-N decreased in the order: PBB-N (r = 0.784** and 0.901**) > KCl-N (r = 0.773** and 0.743**) > acid $KMnO_4$-N (r = 0.575** and 0.651**) ≥ C_{org} (r = 0.591** and 0.531**) ≥ alkaline $KMnO_4$-N (r = 0.394** and 0.548**) > N_{tot} (r = 0.297** and 0.273*). Of all the indices evaluated, PBB-N showed the best correlations with plant parameters as well (r = 0.790** and 0.793** for DMY and PNU, respectively). Based on the highest correlations of PBB-N with biological indices as well as plant responses, we propose PBB-N as an appropriate index of N-availability in the acidic soils of India and other regions with similar soils.

Keywords: biological incubation; chemical extraction; maize; N supply

A reliable estimate of the soil's nitrogen (N)-supplying capacity (NSC) is essential for determining the rate of N fertilizer application required to optimize crop yield and quality, and to minimize the adverse impacts of excess N on environment and farm economy. Among the numerous methods proposed for assaying NSC, biological methods (aerobic and anaerobic incubation) are considered to be the most reliable, as their results are highly correlated with the yield and plant N uptake (PNU). However, since biological methods are highly time consuming and tedious, they are not considered suitable as routine tests. In pursuit of developing a rapid, yet reliable, index of soil N availability, many chemical indices have evolved over time, but no single method has performed consistently enough to receive broad acceptance across a wide range of soils. For example, in a study by Sahrawat (1983), organic C (C_{org}) and total N (N_{tot}) showed strong correlations with PNU (r = 0.82 and 0.84, respectively), whereas, Hussain et al. (1984) reported much weaker correlations between the same (r = 0.38). Similarly, while Gianello and Bremner (1986b) found hot KCl extractable-N (KCl-N) as a good indicator of potentially available-N in soils, the same index was reported as a poor indicator by Hong et al. (1990). Gianello and Bremner (1986b) also recommended phosphate-borate extractable-N as a strong predictor of NSC, whereas Hong et al. (1990) and Sharifi et al. (2007) found them otherwise. Such contrasting performances of soil test methods for available-N in different soils were frequently reported in literature (St. Luce et al. 2011), which reinforces the need of developing region-specific indices for N-availability in a given set of soil, crop and climatic conditions.

In India, C_{org} (Walkley and Black 1934) is frequently used as an index of soil N-availability for making fertilizer-N recommendations. For research purposes, however, 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 which are mostly concentrated in the north-eastern region of India. It is worth noting that the method of Subbiah and Asija (1956) was proposed based on the analysis of 29 soils from India which were mostly alkaline in reaction, and included only one acidic soil from northeast India. It may therefore be hypothesized that the most commonly used alkaline permanganate method may not really be giving a reliable assessment of N availability in acidic soils of India and other regions with similar soils. Moreover, many reports that this method does not provide satisfactory result (Gianello and Bremner 1986b, Elkarim and Usta 2001) further necessitate to evaluate the utility of alkaline $KMnO_4$ method for available-N assessment in such soils. Thus, objective of this study was to determine if the conventionally used alkaline $KMnO_4$-N and C_{org} provides a reliable estimate of N availability in acidic soils of India and, if not, identify an appropriate index of N availability for such soils.

MATERIAL AND METHODS

Soils were collected from 20 representative sites (0–20 cm depth) in the state of Meghalaya, India. These were air-dried, passed through 2-mm sieve (0.5 mm for C_{org}) and analysed for the relevant physico-chemical properties (Table 1) using standard procedures (Page et al. 1982). To assess the correlations between N availability indices and the plant responses, maize (cv. RCM 1-1) was grown in plastic pots containing 7.5 kg of experimental soils with four replications. The plants were harvested at the initiation of tasseling stage, and the dry matter weights were recorded after oven-drying at 70°C for 48 h. Plant N concentration was estimated and the N uptake was subsequently calculated. Kel Plus – N analyzer (Pelican, Chennai, India) was used in soil and plant N analysis.

Soil N-availability indices

Nitrogen released during aerobic incubation (AI-N). A 10-g sample of soil was mixed with 3 g of water-washed 30–60 mesh quartz sand containing 6 mL of water (Keeney and Bremner 1966). The mixture was evenly distributed over the bottom of the beaker and incubated at 30°C under aerobic conditions. After 14 days, the mixture was extracted with 2 mol/L KCl, and N was estimated by distillation in the presence of MgO and Devarda's alloy. The amount of exchangeable NH_4 and NO_3 present in another portion of the soil-sand mixture that was not incubated was also determined. AI-N was estimated as the difference of the two analyses.

Nitrogen released during anaerobic incubation (ANI-N). The method of Waring and Bremner (1964) as modified by Keeney (1982) was followed. A 5-g soil sample was placed with 12.5 mL of water in a test tube that was stoppered and placed in a constant temperature cabinet at 40°C for 7 days. N released was estimated by distilling the contents with 100 mL of 2 mol/L KCl in the presence of MgO. The amount of NH_4-N present in the sample before incubation was also determined. ANI-N was calculated as the difference of the two analyses.

Soil organic carbon (C_{org}) and total nitrogen (N_{tot}). These two soil parameters, as indices of N availability, were determined following wet digestion method (Walkley and Black 1934) and micro-Kjeldahl procedure, respectively.

Alkaline $KMnO_4$ extractable N. Nitrogen released as NH_3 during distillation of 1-g sample of soil with 10 mL of 0.25 mol/L NaOH containing 0.1 g of $KMnO_4$ was estimated as the index of soil N availability (Stanford 1978).

Acidified $KMnO_4$ extractable N. Soil samples (3-g portions) were shaken with 15 mL of 0.05 mol/L $KMnO_4$/0.5 mol/L H_2SO_4 for 1 h and quantitatively transferred to a distillation flask and distilled with 1 mol/L NaOH to estimate N released as NH_3 (Stanford and Smith 1978).

Hot 2 mol/L KCl extractable N (KCl-N). Soil samples (3-g portions) were heated with 20 mL of 2 mol/L KCl for 4 h in a loosely stoppered 100-mL tube placed in a block digester maintained at 100°C (Gianello and Bremner 1986a). The NH_4-N released was determined by steam-distilling the soil-KCl mixture with MgO. Extractable NH_4-N in the soil samples was also determined by steam distillation of another 3-g portion of the soil sample,

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

Sample	Sampling site	pH (1:2.5)	Sand (%)	Clay (%)	CEC [Cmol (P+)/kg]	Base saturation (%)	C_{org} (g/kg)	N_{tot} (mg/kg)	Avail. N (mg/kg)	Avail. P (mg/kg)	Avail. K (mg/kg)
S_1	Nongpoh	4.90	65.44	25.61	12.50	21.28	7.1	1296.1	132.2	6.33	90.0
S_2	Sokhwai	4.50	60.96	29.71	14.80	23.91	17.1	1516.5	249.8	4.93	120.5
S_3	Jirang	4.54	62.77	31.89	14.62	27.91	17.2	1876.8	208.1	3.76	93.9
S_4	Aphrewmer	4.96	68.53	21.46	11.00	23.50	10.24	1488.2	138.8	4.15	100.2
S_5	Nongkrah	4.64	56.11	34.56	14.50	18.97	8.35	1425.2	166.5	4.50	103.1
S_6	Mawphru	4.89	66.34	28.09	13.50	19.70	11.72	1606.1	222.0	5.94	111.0
S_7	Umsning	4.31	51.53	39.04	15.90	18.30	16.55	1421.1	235.2	2.88	125.3
S_8	Mawksiew	5.32	62.29	31.04	14.40	69.22	13.54	1219.2	214.5	4.37	112.7
S_9	Killing	4.60	66.29	28.37	10.63	38.10	15.95	1704.5	203.7	6.68	114.0
S_{10}	Raitong	4.39	49.01	37.65	19.20	13.02	18.2	1281.5	112.0	3.19	82.9
S_{11}	Umiam	4.27	56.96	31.04	14.50	22.89	17.65	1538.2	102.3	2.62	85.0
S_{12}	Nongpoh	4.69	62.77	25.71	18.30	22.13	23.2	1705.3	251.8	7.42	125.8
S_{13}	Umden	4.99	55.63	32.37	14.20	32.61	19.3	2014.0	223.4	6.51	131.0
S_{14}	Umrit	4.79	58.35	32.37	14.90	13.36	16.45	1333.2	184.6	6.51	114.1
S_{15}	Mawhati	4.64	52.96	35.04	10.80	19.54	9.45	1188.5	95.8	3.84	69.7
S_{16}	Klew	4.69	74.08	21.92	9.90	56.56	13.22	1615.6	172.0	2.58	79.9
S_{17}	Myrdon	4.70	63.68	28.37	10.10	19.91	12.5	1316.8	138.0	2.97	98.0
S_{18}	Umsning	5.05	51.63	34.04	10.60	43.39	12.2	1456.9	176.4	6.29	107.4
S_{19}	Mawpun	4.56	50.29	33.71	13.10	45.27	8.6	1296.1	138.9	6.16	97.9
S_{20}	Mynsain	4.79	58.29	31.04	11.10	26.31	7.6	1108.8	120.3	5.59	77.8

but without heating. KCl-N was estimated as the difference between the two extractions.

Phosphate borate buffer (PBB) extractable N (PBB-N). The method involves steam distillation of 4-g soil for 8 min in presence of 40 mL of phosphate-borate buffer solution (pH 11.2) (Gianello and Bremner 1986b). The NH_4-N initially present in the soil was estimated by steam distillation of 4 g soil with 0.2 g MgO and 20 mL of 2 mol/L KCl for 3.3 min. PBB-N was estimated as the difference between the two estimations.

Statistical analysis

Data were analyzed using the SPSS version 16.0 statistical package (SPSS Inc., Chicago, 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 general, soils used in the study showed large variability in N-supplying capacity (NSC) as indicated by the differences in plant parameters including plant N uptake (PNU), which is taken as a more realistic indicator of N availability in soils (Sahrawat 1983, Hussain et al. 1984, Li et al. 2011). PNU ranged from 598 mg/pot (S_{10}) to 1026 mg/pot (S_{12}) which corresponds to 6.7% and 9.0% of the N_{tot} in the respective soils (Table 2). Such variation in soils' NSC is understandable given the wide variations in soil properties including C_{org}, N_{tot} and clay contents (Table 1). So much variability in NSC of soils within a region implies that crop fertilizer-N requirements may also vary greatly on these soils, and this asserts the need of site-specific soil test-based N recommendation, rather than a blanket recommendation for the whole region, to improve the efficiency of applied fertilizers.

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

Sample	Plant response			Indices of available N in soil (mg/kg)							
	DM yield (g/pot)	N content (%)	N uptake (mg/pot)	AI-N	ANI-N	N_{tot}	C_{org} (g/kg)	alk. $KMnO_4$-N	acid $KMnO_4$-N	KCl-N	PBB-N
S_1	72.5	0.87	631	9.74	34.56	1240.4	6.80	107.4	48.6	9.15	10.20
S_2	80.6	0.97	782	15.51	52.65	1461.9	14.59	185.4	102.3	8.40	15.50
S_3	75.6	0.91	688	12.98	35.11	1823.8	15.59	226.0	68.6	11.63	10.52
S_4	73.3	0.88	645	10.68	38.80	1433.4	9.72	121.1	63.8	14.28	13.15
S_5	72.0	0.86	619	10.29	35.18	1370.2	7.83	144.6	52.7	10.18	12.20
S_6	76.8	0.92	707	13.77	41.23	1551.8	10.95	212.6	91.2	11.20	14.18
S_7	83.3	1.00	833	36.95	79.68	1366.0	13.15	182.1	96.8	19.16	34.78
S_8	81.1	0.98	795	24.42	61.18	1163.2	11.40	192.2	89.1	26.39	21.67
S_9	82.6	0.99	818	24.83	65.54	1650.7	13.10	200.3	87.7	7.75	24.40
S_{10}	70.4	0.85	598	9.23	26.18	1225.8	17.42	89.4	46.3	8.36	8.63
S_{11}	71.2	0.86	612	9.42	33.49	1483.7	15.15	77.8	49.2	20.25	10.15
S_{12}	92.4	1.11	1026	78.30	107.66	1651.5	21.60	149.3	107.1	32.20	30.82
S_{13}	88.2	1.06	935	48.71	87.18	1961.6	18.90	229.8	74.2	25.54	32.28
S_{14}	80.7	0.97	783	31.73	74.21	1277.8	15.45	201.0	56.3	23.89	39.10
S_{15}	70.1	0.84	589	9.39	28.59	1132.3	8.96	72.4	51.1	11.44	9.10
S_{16}	78.9	0.95	750	14.66	39.44	1561.4	11.45	154.6	76.2	9.86	13.86
S_{17}	75.5	0.91	687	13.37	38.12	1261.2	10.50	115.4	68.4	8.37	12.77
S_{18}	80.4	0.97	780	15.23	48.75	1402.0	10.05	161.4	81.2	9.48	15.47
S_{19}	74.7	0.90	672	12.69	36.18	1240.4	7.20	97.7	54.6	11.45	12.54
S_{20}	74.6	0.90	671	12.50	35.07	1052.3	6.40	116.9	69.7	13.10	10.22
Mean	77.7	0.93	723	20.72	49.94	1415.6	12.31	151.9	71.8	14.60	17.58

Each datum is the average of four replications. AI-N – available-N through aerobic incubation; ANI-N – available-N through anaerobic incubation

Of the two biological indices of N-availability, on average, values of ANI-N were higher than those of AI-N which 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 (40°C) than in AI (30°C) might have resulted in higher values of mineralised-N (Keeney 1982). Of the four chemical extractants (excluding the soil parameters i.e. N_{tot} and C_{org}), alkaline $KMnO_4$, on average, extracted the highest amount of N followed by acidified $KMnO_4$, whereas the minimum amount was extracted by hot 2 mol/L KCl, resembling closely by phosphate borate buffer (PBB). Similar differences in the extracting ability of these chemicals were also reported by Nayyar et al. (2006) and Li et al. (2011). These differences are considered to result from their differential ability to extract N from different pools of organic-N in soils. Considering such a wide variability in the ability of different extractants to predict potentially-available N in soil, it is imperative to select one which could provide satisfactory correlation with the standard methods (i.e. biological incubations) and more importantly with the plant responses.

Relationships between soil N-availability indices and plant response. Correlations among soil N availability indices were found significant except between N_{tot} and KCl-N (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. Correlations of the chemical indices of soil N-availability with AI-N and ANI-N decreased in the order: PBB-N (r = 0.784** and 0.901**) > KCl-N (r = 0.773** and 0.743**) > acid $KMnO_4$-N (r = 0.575** and 0.651**) ≥ C_{org} (r = 0.591** and 0.531**) ≥ alkaline $KMnO_4$-N (r = 0.394** and 0.548**) > N_{tot} (r = 0.297** and 0.273*).

Table 3. Correlation coefficients for the relationships between the biological and the chemical indices of N availability

	AI-N	ANI-N	N_{tot}	C_{org}	Alk. $KMnO_4$-N	Acid $KMnO_4$-N	KCl-N	PBB-N
AI-N	1	–	–	–	–	–	–	–
ANI-N	0.946**	1	–	–	–	–	–	–
N_{tot}	0.297**	0.273*	1	–	–	–	–	–
C_{org}	0.591**	0.531**	0.552**	1	–	–	–	–
Alk. $KMnO_4$-N	0.394**	0.548**	0.422**	0.330**	1	–	–	–
Acid $KMnO_4$-N	0.575**	0.651**	0.244*	0.307**	0.603**	1	–	–
KCl-N	0.773**	0.743**	ns	0.455**	0.255*	0.291**	1	–
PBB-N	0.784**	0.901**	0.236*	0.465**	0.587**	0.468**	0.672**	1

*P < 0.05; **P < 0.01; ns - not significant. AI-N – available-N through aerobic incubation; ANI-N – available-N through anaerobic incubation

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**). Among the chemical indices, best correlation with PNU was shown by PBB-N (r = 0.793**) followed by acid $KMnO_4$-N (r = 0.739**). Alkaline $KMnO_4$-N correlated slightly better (r = 0.625**) than the KCl-N (r = 0.604**). Of all the indices, N_{tot} and C_{org} showed the least correlations with PNU, with the latter being better correlated (r = 0.516**) than the former (r = 0.350**). Correlations of the dry matter yield and %N with various indices showed similar trend and strength as with PNU.

Significant correlations observed between most of the indices are expected since most of the extractants draw heavily on soil organic matter N pools (Nayyar et al. 2006). However, their differential ability to extract N from different pools may have resulted in hugely different levels of correlations between them. The better reliability of biological indices as compared to chemical ones 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). However, since these methods are tedious and time consuming, an alternative chemical index of soil N-availability, which correlates strongly with the results of incubation methods and more importantly with plant responses, is preferred for routine soil analysis. Among all the six chemical indices, PBB-N showed consistently higher correlations with the results obtained by biological methods as well as with plant parameters. This implies that PBB-N deserves consideration as a routine test for available N in acidic soils of India. The usefulness of PBB-N as a reliable index of soil N-availability was established by many studies involving a wide range of soils and crops (Gianello and Bremner 1986a,b, Hong et al. 1990, Elkarim and Usta 2001).

Two soil parameters namely, N_{tot} and C_{org} were also evaluated as indices of soil N-availability, but their correlations with either the biological indices or the plant responses were not promising enough to be taken as a reliable predictor of N-availability. In India, C_{org} is frequently used by soil testing laboratories as an index of N availability. However, results of our study on acidic soils of northeast India, supported by similar results from alkaline soils of northwest India (Nayyar et al. 2006), suggest that an alternative chemical index with better predictive ability than the C_{org} should be used for accurate prediction of plant-available N in Indian soils.

Table 4. Correlation coefficients for the relationships between plant parameters and the indices of soil N availability

	AI-N	ANI-N	N_{tot}	C_{org}	Alk. $KMnO_4$-N	Acid $KMnO_4$	KCl-N	PBB-N
Dry matter yield	0.885**	0.925**	0.348**	0.522**	0.633**	0.761**	0.599**	0.790**
N concentration	0.876**	0.928**	0.313**	0.490**	0.631**	0.759**	0.607**	0.789**
N uptake	0.876**	0.920**	0.350**	0.516**	0.625**	0.739**	0.604**	0.793**

*P < 0.05; **P < 0.01. AI-N – available-N through aerobic incubation; ANI-N – available-N through anaerobic incubation

It is also noteworthy that the alkaline $KMnO_4$-N, which (in addition to C_{org}) happens to be the most widely used index of soil N-availability in India, although significantly correlated, did not yield satisfactory results compared to the other chemical indices such as PBB-N and acid $KMnO_4$-N. Many studies reported that alkaline $KMnO_4$ method does not provide satisfactory results (Stanford 1978, Gianello and Bremner 1986b, Elkarim and Usta 2001). Our results, which further consolidate these previous findings, suggest that PBB-N, rather than the alkaline $KMnO_4$-N, can be better used as a reliable index of soil-N availability, particularly in the acidic soils of India.

To conclude, of the six chemical indices of soil N-availability evaluated in this study, PBB-N showed consistently higher correlations with the results obtained by the standard incubation methods as well as with plant parameters. The two commonly used indices of N availability in India, namely C_{org} and alkaline $KMnO_4$-N did not perform satisfactorily when tested for acidic soils. We, therefore, on the basis of our results, propose PBB-N as a better alternative index of N-availability for the acidic soils of India and the other regions with similar soils.

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