



Conservation agriculture influences soil nitrogen availability in the lower Indo-Gangetic Plains

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Abstract

Background and aims Information on the effect of conservation agriculture (CA) on N availability in soil is inconsistent. Estimation of available N using conventional analytical methods may not be suitable due to changes in N forms in soils under CA. The study aimed to assess the impact of CA on N availability in soil and to evaluate estimation methods for plant-available N under CA.

Methods A field experiment was conducted involving fifteen treatments comprising three tillage operations and five rice residue + nutrient management practices

with three cropping systems [rice-maize-cowpea (RMaC), rice-mustard-black gram (RMuB) and rice-cauliflower-rice (RCR)] in alluvial soils of the lower Indo-Gangetic Plains. Availability of N in surface soil layers was assessed using neutral phosphate buffer (PB), calcium chloride (CC), sodium bicarbonate (SB) and alkaline permanganate (PP) methods after three years.

Result The amount of available N extracted by the four methods followed the order SB > PB > PP > CC. Zero tillage with 50% residue + 100% NPK and 100% residue + 75% NPK resulted in ~3 to 20% and 3 to 12%, respectively, higher available N in soils over other CA treatments for RMaC and RMuB cropping

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systems. In contrast, reduced tillage with 50% residue + 100%NPK had ~1 to 13% higher available N for RCR cropping system.

Conclusion This work offers choice of efficient CA practices with higher plant available N in soils. Novel multi-criteria-based technique identified PB as the best method in estimating available N in soils under CA with rice-based cropping systems.

Keywords Available N · Phosphate buffer · Potentially mineralizable N · Residue management · Tillage operation

Introduction

Conservation agriculture (CA) is one of the resource conservation strategies which minimizes the impact of farming on the environment (Nayak et al. 2022; Saurabh et al. 2021) and provides yield benefits in a sustainable manner over the conventionally managed systems (Kassam et al. 2019; Wu et al. 2021). It entails three principles namely, (i) limited or zero soil disturbances (i.e., minimum tillage or no-tillage), (ii) increased crop residue retention to ensure maximum soil cover, and (iii) diverse crop rotation (Page et al. 2020; Patel et al. 2023). Adoption of CA in intensive rice-based cropping systems in the Indo-Gangetic Plains (IGP) of South Asia is increasing (Kassam et al. 2019). Non-puddled transplanting of rice with zero tillage (ZT) or reduced tillage (RT) is proven to be a novel crop establishment practice designed for CA (Das et al. 2020; Kader et al. 2022). Crop residue retention or incorporation at the soil surface under CA increases soil organic C and conserves soil and water for sustaining crop production (Kumar et al. 2023). This also curbs the menace of crop residue burning and attendant losses of C and N, native soil organic matter and microbial activity (Alam et al. 2020; Saurabh et al. 2021). Therefore, physical, chemical and biological soil properties are reportedly improved with ZT or RT, residue management and diversified crop rotation under CA (Faiz et al. 2022; Kumar et al. 2021; Nandan et al. 2019).

As much as 90–95% of soil N is in organic forms (Liu et al. 2018), and their dynamics and transformations largely depend on addition of organics by different farming practices (Mukherjee et al. 2023; Sarkar et al. 2023). Microbial decomposition of crop residues releases plant nutrients (Khan et al. 2024). CA

regulates N cycling (Badagliacca et al. 2021; Topa et al. 2021) by influencing mineralization and immobilization in soil (Alam et al. 2020; Bhattacharyya et al. 2019; Verhulst et al. 2013). This, in turn, causes a change in total, inorganic and organic N fractions with CA over CT (Kumar et al. 2021; Parihar et al. 2018; Verhulst et al. 2013). Repeated disturbance to soils with CT and incorporation of crop residues along with basal N fertilization, higher soil temperature and higher microbial activity may account for higher N availability during the early years of cultivation (Badagliacca et al. 2021; Yadvinder-Singh et al. 2015). However, the depletion of available N in soil over time under CT suggests that either the readily mineralizable N fraction is reduced in successive years or there are greater N losses with CT over CA (Alam et al. 2020). Although ZT or RT is effective in reducing surface losses of N, effects of ZT/RT with and without residue retention or incorporation on N availability in soils are ambiguous (Yadvinder-Singh et al. 2015; Verhulst et al. 2013). Despite, many studies comparing the impact of CA on N cycling, N utilization and crop productivity (Alam et al. 2020; Bhattacharyya et al. 2019; Jat et al. 2018; Kader et al. 2022; Nayak et al. 2022; Wu et al. 2021), limited information is available regarding the impact of different intensities of CA including varied residue and nutrient management practices for different cropping systems on N availability in soil.

Several methods, such as alkaline permanganate (Subbiah and Asija 1956), hydrochloric acid or sulphuric acid (Peterson et al. 1960), alkaline calcium hydroxide (Prasad 1968), hot-water (Keeney and Bremner 1966) and cold dilute barium hydroxide solution (Setatou and Simonis 1996) are commonly used for estimating available N status in conventionally managed soils. However, their suitability in organic-rich CA soils has received limited attention. Availability of N in soil was also estimated considering the amount of N mineralized during incubation of soil at 30 °C under field moisture conditions (Keeney and Nelson 1982; Mukherjee et al. 2021a), since N estimated by this method is correlated with plant uptake and crop yield (Mukherjee et al. 2021a). However, this method takes several weeks to determine available N content in soil. Availability of carbonaceous materials in soils under CA enriches potentially mineralizable N (PMN) fractions, which contribute nearly 40–60% of the organic N. As PMN fractions

are primarily composed of easily mineralizable protein or protein-like N compounds, these are vulnerable to quick mineralization for producing available N for plant uptake (da Silva et al. 2019; Saha and Mandal 2011). Moreover, lignin, tannin, quinone-bound protein-like compounds, amino sugars and other organic N complexing with polyvalent cations like Fe and Al may contribute to plant N availability in soil (da Silva et al. 2019). Conventional methods for estimating plant available N in soil measure only the labile soil N including the most dynamic fractions, which hardly include PMN fractions in organic-rich soils (da Silva et al. 2019; Liptzin et al. 2023; Mukherjee et al. 2021b). Therefore, we hypothesized that methods typically used for estimating available N in soils under conventional systems may not be equally applicable in soils under CA. Several N extraction methods like 1/15 M phosphate buffer (PB; Matsumoto et al. 2000a), 0.01 M calcium chloride (CC; Appel and Mengel 1992) and 0.01 M sodium bicarbonate (SB; Bradford 1976) are expected to have the capacity to extract protein-like and easily mineralizable organic N compounds (e.g., amino acid, amino sugar, hydrolyzable ammonium) from soils. Most of these methods extract easily mineralizable N through ion-exchange or solubilization mechanisms (Matsumoto and Ae 2004). An examination of these methods (PB, CC and SB) for estimating plant available N content in soils under CA as compared to the most common method i.e., alkaline permanganate method (PP; Subbiah and Asija 1956) warrants further examination. Therefore, in the present study, after three years of continuous CA under three rice-based cropping systems in the alluvial soil of the lower IGP of eastern India, we assessed: (i) effects of CA on N availability in soils, and (ii) the suitability of methods for estimating available N in soils for cowpea, black gram, and rice grown under CA.

Materials and methods

Study site characteristics

A field experiment was initiated in the *Kharif* (rainy) season of 2018 at Balindi Farm under the Centre for Advanced Agricultural Science and Technology on Conservation Agriculture, Bidhan Chandra Krishi Viswavidyalaya (22° 57' 46" N, 88° 31' 48" E; 9.75

m above mean sea level). The site experiences subtropical humid climate with an average annual rainfall of 1560 mm and mean annual minimum and maximum temperatures of 21.3 and 33.2 °C, respectively. Its soil was classified as alluvial, *Inceptisol* (Soil Survey Staff 2003) with clay loam texture. Bulk density [core sampling method (Blake and Hartge 1986)], pH and electrical conductivity [in 1:2.5 (w/v) soil-water suspension (Jackson 1973)], 0.167 M K₂Cr₂O₇ oxidizable organic C (Walkley and Black 1934), alkaline 0.32% KMnO₄ extractable N (Subbiah and Asija 1956), 0.5 M NaHCO₃ (pH 8.5) extractable P (Olsen et al. 1954) and neutral 1.0 M NH₄ acetate extractable K (Hanway and Heidel 1952) content of the initial soil at 0–0.20 m depth were 1.52 g cm⁻³, 7.4, 0.24 dS m⁻¹, 7.8 g kg⁻¹, 222 kg ha⁻¹, 25 kg ha⁻¹ and 297 kg ha⁻¹, respectively.

Experimentation

The field experiment was conducted with three different rice-based cropping systems namely, rice-maize-cowpea (RMaC), rice-mustard-black gram (RMuB) and rice-cauliflower-rice (RCR) in a split-plot design. Each cropping system had three main treatments of tillage intensity viz., conventional tillage (CT), zero tillage (ZT) and reduced tillage (RT). Every main treatment was subdivided into five sub-treatments consisting of a combination of inorganic N, P and K fertilization and rice residue retention at various rates (Table 1). This resulted in fifteen (3×5=15) tillage and residue+nutrient combinations with each cropping system with a plot size of 10 m × 20 m each, with three replications.

Only on completion of three cycles (years) of cultivation of the systems with those 15 treatments, we took observations for the present study with the premise that by this time, the systems attained a quasi-equilibrium state and bear the signature of a true CA in respect of its N-cycling (Meena et al. 2015; Timisina et al. 2006). As such, we claimed that the methods tested for estimating available N in soils actually captured the typical N-dynamics in soils under a true CA system. The use of rice-based systems also helped because it not only shows a minimum effect towards the imposed treatments but also is quite efficient in obscuring the system's small and temporal variations due to prolonged submergence for rice cultivation. Again, we retained only the residues of *Kharif* rice

Table 1 Treatments of tillage, crop residue and fertilizer used in the experiment

Cropping systems	Tillage intensities	Residue* doses	Nutrient doses	Treatment symbols	
Rice-maize-cowpea	Conventional tillage:	0% residue	100% NPK	CT+0R+100NPK	
Rice-mustard-black gram	two primary and two secondary tillage operations	100% residue	50% NPK	CT+100R+50NPK	
Rice-cauliflower-rice		100% residue	75% NPK	CT+100R+75NPK	
		50% residue	100% NPK	CT+50R+100NPK	
		50% residue	75% NPK	CT+50R+75NPK	
		Reduced tillage:	0% residue	100% NPK	RT+0R+100NPK
		one primary and one secondary tillage operations	100% residue	50% NPK	RT+100R+50NPK
			100% residue	75% NPK	RT+100R+75NPK
			50% residue	100% NPK	RT+50R+100NPK
			50% residue	75% NPK	RT+50R+75NPK
		Zero tillage:	0% residue	100% NPK	ZT+0R+100NPK
	without any primary and secondary tillage operations	100% residue	50% NPK	ZT+100R+50NPK	
		100% residue	75% NPK	ZT+100R+75NPK	
		50% residue	100% NPK	ZT+50R+100NPK	
		50% residue	75% NPK	ZT+50R+75NPK	

NPK recommended dose of N, P and K fertilizers using urea and customized 10.0-11.4-21.7 (N-P-K) grade fertilizer

*Rice residue retention for the succeeding *Rabi* crops

(because of socio-economic compulsion of the farmers of the region) for the last three years for all the three cropping systems following farmers' practices. To perceive the maximum effect of CA on N mineralization in the soil, as well as ascertaining a suitable N availability index, we sampled our experimental soils from the fields after harvesting of the *Rabi* crops for necessary analysis. Succeeding crops viz., cowpea, black gram and summer (*Boro*) rice were chosen as the test crops. Details of the crop management practices of the test crops under three cropping systems are described in Table 2.

Soil and plant sampling

Soil samples were collected at 0–0.20 m depth after harvesting of the third *Rabi* crop i.e., after maize for RMaC, mustard for RMuB and cauliflower for RCR cropping systems from each of the 15 selected tillage and residue+nutrient management treatments with three replications. Soil samples were then air-dried, passed through a 2-mm nylon sieve and subsequently, stored in polyethylene bottles for future analysis. Plant samples (grain and stalk) of succeeding crops, cowpea, black gram and *Boro* rice were collected at harvest from RMaC, RMuB and RCR cropping systems, respectively. Plant samples were washed with

running tap water followed by 0.01 M HCl and distilled water and dried in a hot-air oven at 60 °C for 48 h. Dried plant samples were ground to fine powder using a mechanical grinder for further analysis. Grain yield was recorded for the studied crops at harvest.

Analysis of soil and plant samples

The soil samples were extracted for available N by four extraction methods viz., 1/15 M phosphate buffer (PB; Matsumoto et al. 2000a; Mukherjee et al. 2021a, b), 0.01 M calcium chloride (CC; Appel and Mengel 1992) and 0.01 M sodium bicarbonate (SB; Bradford 1976; Michrina et al. 1982) and alkaline 0.32% KMnO₄ (PP; Subbiah and Asija 1956) (Supplementary information Table S1). The first three methods (PB, CC and SB) included inorganic N plus PMN fractions composed of protein-like and easily mineralizable organic N compounds such as amino acid, amino sugar, and hydrolyzable ammonium (Appel and Mengel 1992; Bradford 1976; Matsumoto et al. 2000a). Inorganic N (NH₄⁺-N+NO₃⁻-N) in these extracts was estimated using 2 M KCl solution at 1:10 (extract to KCl solution v/v) ratio. The NH₄⁺-N was estimated by steam distillation with a mild-oxidizing agent (MgO) in the Kjeldahl distillation unit and NO₃⁻-N by reduction with Devarda's alloy

Table 2 Details of the management practices followed for the test crops

Particulars	Cowpea	Black gram	Boro rice
Cropping system	Rice-maize-cowpea	Rice-mustard-black gram M	Rice-cauliflower-Boro rice
Crop variety	<i>Krishni Nidhi</i>	<i>Goutam</i>	<i>Ajit</i>
Crop sowing/planting	Seeds were sown in undisturbed soils using zero-till-seed drill for zero-till-seed plots; whereas in the cases of reduced-tilled and conventionally-tilled plots, multi-crop seed cum fertilizer drill was used. The machine penetrated the target soil surface layer, opened a slot for seeding and then, placed the seed at the desired depth (3–5 cm depth) within the slot along with basal dose of fertilizers for all the tillage conditions. For conventionally-tilled and reduced-tilled rice, seeds were sown in seedbeds with multi-crop seed cum fertilizer drill and transplanted manually to the main field after 21 days of seed sowing; whereas in the case of zero-till-seed plots, seeds were directly sown to the undisturbed soil using zero-till-seed drill.		
Fertilizer management	20 kg N, 26 kg P and 72 kg K ha ⁻¹ applied as basal in the form of urea and customized 10.0-11.4-21.7 (N-P-K) fertilizer	20 kg N, 17.5 kg P and 48 kg K ha ⁻¹ applied as basal in the form of urea and customized 10.0-11.4-21.7 (N-P-K) fertilizer	120 kg N, 26 kg P and 72 kg K ha ⁻¹ in the form of urea and customized 10.0-11.4-21.7 (N-P-K) fertilizer. ½ N and full amount of P and K applied as basal, ¼ N each at 25 and 55 days after transplanting.
Residue management	No residue of the previous crop was added there for cowpea, black gram and Boro rice. The residue of the <i>Kharif</i> rice obtained from different treatments was retained for the succeeding <i>Rabi</i> crops maize, mustard and cauliflower at the rate of 100, 50 and 0% of the residue produced.		
Irrigation management	Irrigation was done at 50% moisture deficit in soil to maintain sufficient moisture for crops.		6 to 7 irrigations each with 5–6 cm water were applied depending upon the amount and distribution of rainfall received during rainy season
Intercultural operations	Weeds in the experimental plots were controlled by applying pre-emergence and post-emergence herbicides. In addition, glyphosate (41% SL) was applied to zero-till-seed plots at 750 g a.i. ha ⁻¹ 7–10 days before crop sowing. Diseases and pests were controlled following standard management practices.		

(Keeney and Nelson 1982). Amount of this inorganic N was then subtracted from PB, CC and SB extractable N fractions and the remaining amounts (mostly PMN including protein-like and easily mineralizable organic N fractions) were considered as the PB, CC and SB extractable available N in soils, respectively. Total N and easily mineralizable fractions of soil organic N, such as amino acid, amino sugar and hydrolyzable ammonium, were analyzed following the standard procedures (Bremner and Keeney 1965; Stevenson 1996). Inorganic N content in soils was estimated using 2 M KCl solution at 1:10 (soil to solution w/v) ratio following the same procedure as mentioned earlier. The amount of N fractions in soils was expressed in kg ha^{-1} , which was obtained by multiplying soil N content with the depth and bulk density of the experimental soils. Nitrogen concentration in stalk and grain was analyzed by micro-Kjeldahl digestion and distillation method (Page et al. 1982). Uptake of N was calculated as the product of N concentration and dry weight of plant biomass (stalk and grain).

Suitability of methods for extraction of available N in soil

To identify the most effective method(s) for estimation of available N in soil a multi-criteria assessment of the N extraction methods was used, based on the criteria below:

Criteria 1: Relationships of the amount of available N extracted by different methods and plant parameters (grain and stalk N contents, N uptake by crop and crop yield) by correlation study, which is the conventional way of screening a suitable method; *Criteria 2:* Relationships of the amount of N extracted by different methods and easily mineralizable organic N fractions (hydrolyzable N fractions viz., amino acid, amino sugar and hydrolyzable ammonium); *Criteria 3:* Relationships of the amount of N extracted by different methods and inorganic N; *Criteria 4:* Responsiveness of different methods to total soil N content computed through linear regression model between total N (kg ha^{-1}) of different management practices and the amount of N extracted by the methods compared (kg ha^{-1}); *Criteria 5:* The extent of variability among the methods was calculated from the coef-

ficient of variation (%) i.e., $\text{CV} = \text{SD}/\text{mean} \times 100$, where SD is the standard deviation of the extractable N by any method. Sensitivity analysis (S) was also done for all the methods by computing the ratio between the maximum and minimum values recorded with each method.

Finally, we assessed suitability of different methods for extraction of available N in soil under CA by considering all the above criteria. The corresponding values of all five criteria indicated a particular criterion's relative contribution to finding the suitable method(s). The methods were then ranked from 1 to 4 by scoring for the best choice as 1. For example, the methods showed the highest correlation coefficients for criteria 1 to 3, the highest slope value (m) for criteria 4 and the highest 'CV' and 'S' values for criteria 5 were ranked the highest i.e., scored 1. We calculated the mean score of the five criteria to examine the suitability of the methods for individual cropping system. Overall, suitability of methods was examined from the mean score of the five criteria across cropping systems.

Statistical analysis

The amount of available N, inorganic N, easily mineralizable organic N fractions: amino acid, amino sugar and hydrolyzable ammonium in soils and plant parameters such as grain and stalk N contents, N uptake by crop and crop yield (response variables) were subjected to analysis of variance using the generalized linear model on split-plot design to determine impact of tillage, residue + nutrient, cropping system and their interactions (fixed effects). Differences among tillage operations, residue + nutrient treatments and cropping systems and tillage plus residue + nutrient combinations were compared at 5% probability level through Duncan's multiple range test using Statistical Package for the Social Sciences (SPSS) software (version 20.0). Simple linear correlations of available N content in soils estimated by different methods were performed with plant parameters, easily mineralizable organic N fractions and inorganic N. Linear regression equations taking total N as the fixed factor and the amount of available N by different methods as the random variables were developed. To delineate the variation and contribution of available N content in soils estimated by different

methods to crop performance and easily mineralizable N fractions, principal component analysis (PCA) was executed using Statistical Tool for Agricultural Research (STAR) package (version: 2.0.1; <http://bbi.irri.org/trainings/biom206>) created in the R software interface (R Core Team 2021).

Results

Nitrogen availability in soils

N extractability of different methods

Plant available N contents in soils varied significantly ($p < 0.05$) with N extraction methods. The content of PB, CC, SB and PP extractable N in surface soil (0–0.20 m depth) varied from 300 to 369, 242 to 314, 324 to 392 and 273 to 337 kg ha^{-1} with mean

values of 334, 277, 354 and 304 kg ha^{-1} , respectively, across tillage operations, residue + nutrient management practices and cropping systems after three years (Fig. 1). On average, the order of the methods with respect to the amount of available N extraction was $\text{SB} > \text{PB} > \text{PP} > \text{CC}$.

Effect of CA on N availability in soils

Tillage operations had a significant ($p < 0.05$) influence on available N content in soils. ZT and RT were associated with a higher amount of available N in soils than CT under the RMaC system. In contrast, ZT was associated with the most available N among the tillage operations under the RMuB system. In the case of RCR system, RT had higher available N than CT or ZT (Supplementary information Fig. S1). Average magnitude of increase in available N content with ZT and RT was ~6% and 2%, respectively,

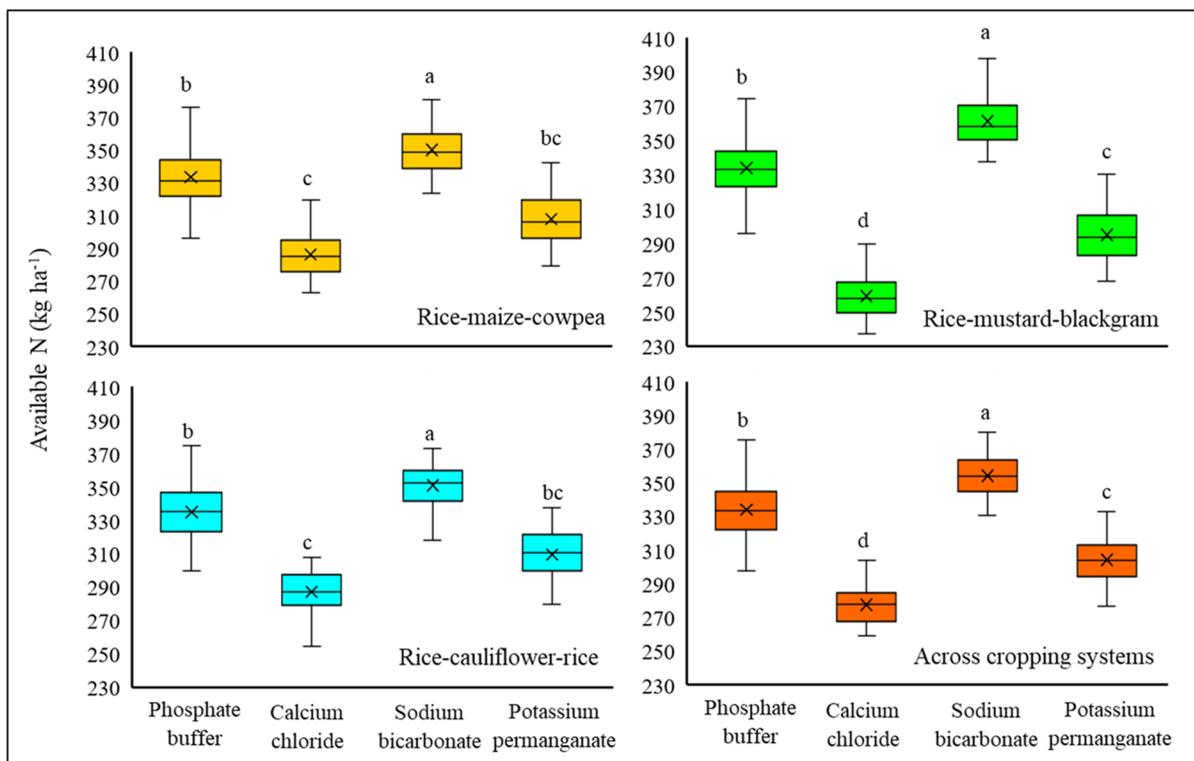


Fig. 1 Amount of available N in soils (0–0.20 m depth) with different cropping systems as extracted by the four methods selected for comparison. Columns labeled with different lowercase letters are significantly different by Duncan's multiple range test ($p = 0.05$). The symbol cross (x) indicates mean

value; horizontal line in the box indicates median; length of the box indicates interquartile range; upper and lower whiskers indicate the extent of maximum and minimum values of the range of the data set, respectively

over CT across RMaC and RMuB systems. Contrarily, in RCR system, RT had ~3 and 4% higher available N over CT and ZT, where the effect of CT and ZT was at par. Residue + nutrient management practices also significantly ($p < 0.05$) altered N availability in soils. Among the residue + nutrient treatments, 50R+100NPK followed by 100R+75NPK were associated with higher amount of available N (~2 to 9 and 3 to 7% higher, respectively, over the others) across the tested methods and cropping systems. The effect of cropping system was nonsignificant on available N content in soils. On average, available N content was 319, 312 and 321 kg ha^{-1} with RMaC, RMuB and RCR cropping systems, respectively.

On average, ZT+50R+100NPK had ~3 to 20% higher available N in soils than the other CA treatments with RMaC and RMuB systems followed by ZT+100R+75NPK treatment (~3 to 12% higher) (Fig. 2). In the case of RCR system, RT+50R+100NPK retained ~1 to 13% higher available N in soil among the CA treatments. In contrast, CT+100R+50NPK recorded the lowest value of available N among the CA treatments across cropping systems (~296 kg ha^{-1} ; 3 to 13% lower than the others).

Suitability of methods for assessment of available N in soils

Relationships between available N and plant parameters

Different levels of CA practices improved the N uptake by cowpea, black gram, and *Boro* rice by 23, 23 and 6%, respectively over CT after three years of experimentation. The productivity of cowpea and black gram was increased by 24 and 27%, respectively with CA treatments, while that of *Boro* rice was dropped by 4% of the CT (Supplementary information Figs. S2 and S3). Available N content in soils, which was extracted by the four different methods, showed significant relationships with plant parameters of cowpea, black gram and *Boro* rice grown in RMaC, RMuB and RCR cropping systems, respectively. The values of correlation coefficients indicated that PB had the strongest relationships with grain and stalk N concentrations, N uptake and grain yield of different crops (Table 3). Next to PB, PP showed stronger

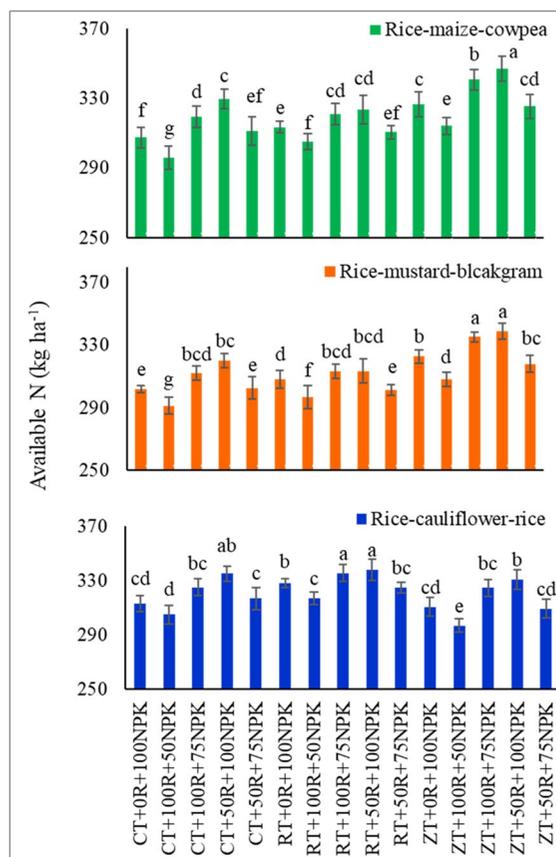


Fig. 2 Effect of 15 treatments including conservation agriculture on available N content in soils across extraction methods under different cropping systems. CT, conventional tillage; RT, reduced tillage; ZT, zero tillage; R, residue; NPK, recommended dose of N, P and K fertilizers; columns labeled with different lowercase letters are significantly different by Duncan's multiple range test ($p=0.05$). The error bar represents the standard error of the mean

relationships with the plant parameters followed by CC and SB in decreasing order of magnitude.

Relationships between available N and mineralizable soil N fractions

The amount of mineralizable N fractions (viz., hydrolyzable N, amino acid, amino sugar and hydrolyzable ammonium) and inorganic N was significantly higher (~2, 7, 13, 10 and 16%, respectively) with different CA practices over the conventional one (Supplementary information Fig. S5). As extracted by different methods, available N content showed significant positive relationships with

Table 3 Linear correlation coefficients (r) between the amount of available N in soils as estimated by different methods and plant parameters (grain N, stalk N, N uptake, grain yield) with different cropping systems

Methods	Grain N	Stalk N	N uptake	Grain yield
Rice-maize-cowpea				
Phosphate buffer	0.88**	0.81**	0.95**	0.93**
Calcium chloride	0.77**	0.85**	0.74**	0.66**
Sodium bicarbonate	0.72**	0.70**	0.75**	0.71**
Potassium permanganate	0.85**	0.81**	0.90**	0.86**
Rice-mustard-black gram				
Phosphate buffer	0.79**	0.65**	0.89**	0.88**
Calcium chloride	0.72**	0.64*	0.61*	0.59*
Sodium bicarbonate	0.61*	0.66**	0.66**	0.63*
Potassium permanganate	0.80**	0.79**	0.82**	0.77**
Rice-cauliflower-rice				
Phosphate buffer	0.93**	0.91**	0.92**	0.83**
Calcium chloride	0.69**	0.71**	0.80**	0.80**
Sodium bicarbonate	0.78**	0.75**	0.77**	0.69**
Potassium permanganate	0.92**	0.92**	0.92**	0.83**

* and ** indicate correlations are significant at the 0.05 and 0.01 levels, respectively

the amount of these mineralizable N fractions and inorganic N in soils (Table 4). Among the methods of available N extraction, PB secured the strongest correlations with the mineralizable N fractions under all the cropping systems, followed by PP. In

contrast, such relationships involving SB and CC were weak. On the other hand, PP established the strongest relationships with the inorganic N followed by PB, SB and CC in decreasing order of magnitude for all the cropping systems (Table 4).

Table 4 Linear correlation coefficients (r) between the amount of available N in soils as estimated by different methods with the amount of easily mineralizable N fractions and inorganic N in soils with different cropping systems

Methods	Major contributory organic N pools to plant N availability				Inorganic N
	Hydrolyzable N	Amino acid	Amino sugar	Hydrolyzable ammonium	
Rice-maize-cowpea					
Phosphate buffer	0.90**	0.89**	0.88**	0.82**	0.92**
Calcium chloride	0.77**	0.58*	0.74**	0.60*	0.76**
Sodium bicarbonate	0.74**	0.67**	0.81**	0.66**	0.80**
Potassium permanganate	0.87**	0.83**	0.89**	0.79**	0.96**
Rice-mustard-black gram					
Phosphate buffer	0.65**	0.90**	0.89**	0.87**	0.86**
Calcium chloride	0.54*	0.46	0.66**	0.62*	0.69**
Sodium bicarbonate	0.41	0.64*	0.79**	0.70**	0.86**
Potassium permanganate	0.61*	0.77**	0.84**	0.81**	0.93**
Rice-cauliflower-rice					
Phosphate buffer	0.82**	0.93**	0.80**	0.83**	0.94**
Calcium chloride	0.85**	0.61*	0.74**	0.69**	0.60*
Sodium bicarbonate	0.72**	0.68**	0.70**	0.70**	0.73**
Potassium permanganate	0.81**	0.91**	0.77**	0.83**	0.97**

* and ** indicate correlations are significant at the 0.05 and 0.01 levels, respectively

Responsiveness of available N extraction methods to total soil N

We calculated the responsiveness of N extraction methods of available N to total N in soils (mean total N content was 2643, 2702 and 2622 kg ha⁻¹ for RMaC, RMuB and RCR systems, respectively) following the linear regression models. Among the methods, PB and PP showed higher responses to total N, as evident from the greater slope of the linear regression lines, whereas such responses were weak for SB and CC for all the cropping systems (Fig. 3).

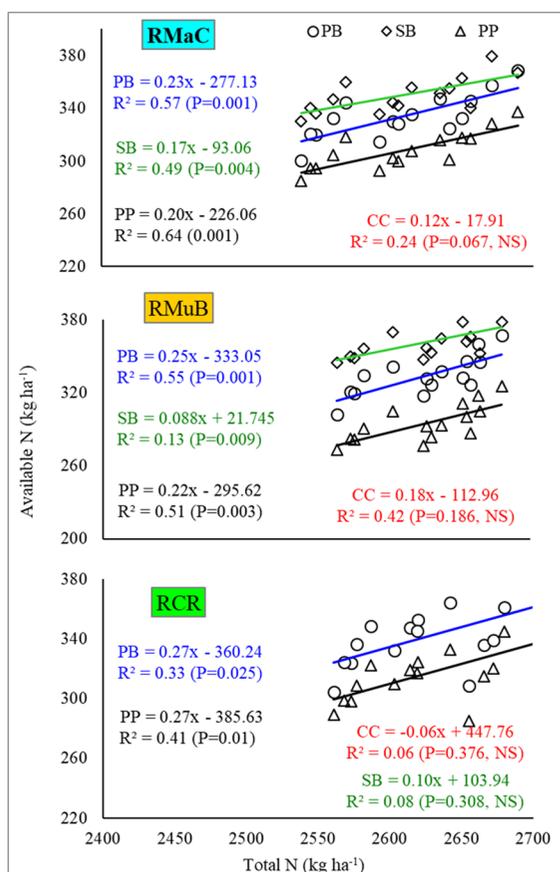


Fig. 3 Responsiveness of extractants to total N in soils computed through linear regression model under different cropping systems. PB, phosphate buffer; CC, calcium chloride; SB, sodium bicarbonate; PP, potassium permanganate; RMaC, rice-maize-cowpea; RMuB, rice-mustard-black gram; RCR, rice-cauliflower-rice; NS indicates non-significant regression

Extent of variability and sensitivity of available N extraction methods

Among the four methods, PB and PP showed higher CV in extracting soil available N under CA practices (Fig. 4). Of the two, PB had the highest values of CV with RMaC (5.2) and RCR (5.2) cropping systems; whereas PP had it with RMuB (5.4) system. In the case of sensitivity analysis, all the methods showed a similar range of values (1.1 to 1.2) with no significant variation among the methods with all the cropping systems.

Principal component analysis

Results of the PCA showed that the first three principal components accounted for ~87% of the total variation in soil available N extracted by different methods, crop parameters and N fractions in soils under CA practices (Supplementary information Table S2; Fig. 5). The first principal component explained ~73% of the total variation with the highest loading on PB; while the second and third principal component explained only ~7 and 6%, variability and the highest loading was on SB and CC, respectively.

Ranking of available N extraction methods

Ranking of available N extraction methods based on their performance in each suitability criterion showed that PB had the lowest mean score for all the cropping systems and ranked first among the four methods

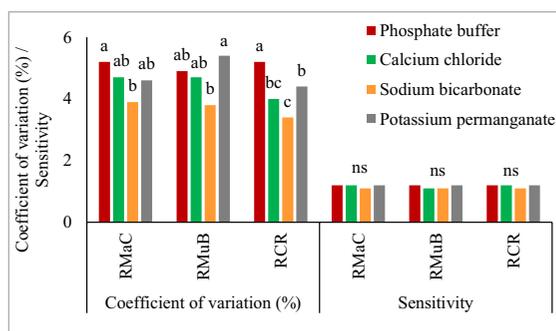
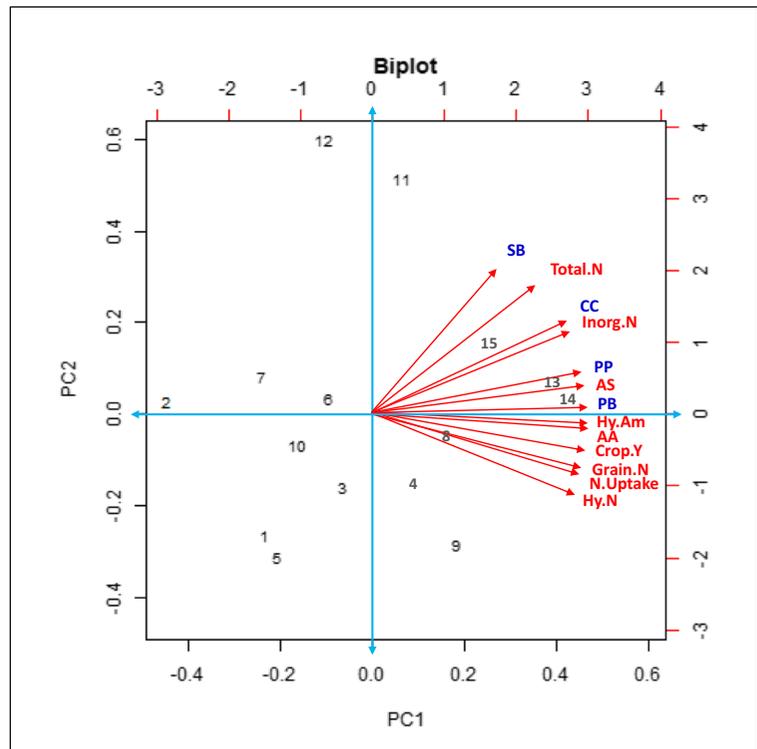


Fig. 4 Magnitude of variability and sensitivity of the methods for estimation of available N in soils. Sensitivity, ratio of maximum and minimum values; CV, coefficient of variation (%); RMaC, rice-maize-cowpea; RMuB, rice-mustard-black gram; RCR, rice-cauliflower-rice; columns labeled with different lowercase letters are significantly different by Duncan's multiple range test ($p=0.05$)

Fig. 5 Evaluation of different available N extraction methods for explaining crop performance and contributory N fractions by principal component analysis biplot. PB, 1/15 M phosphate buffer; CC, 0.01 M calcium chloride; SB, 0.01 M sodium bicarbonate; PP, 0.32% potassium permanganate; Total.N, total nitrogen; Inorg.N, inorganic nitrogen; Hy.N, hydrolyzable nitrogen; AA, amino acid; AS, amino sugar; Hy.Am, hydrolyzable ammonium; Crop.Y, crop yield; Grain.N, N concentrations in grain, N.Uptake, nitrogen uptake by plants; PC, principal component



(Table 5); while PP method was the next best in the ranking. Overall, the order of suitability of methods for estimating available N in soils under CA was $PB > PP > SB > CC$.

Discussion

Efficiency of methods for extraction of available N in soils under CA

Available N extraction methods differed in estimating N availability in soils due to the differences in their chemical composition (associated ions and pH), mechanism of extraction and the nature of N compounds present in soil. Application of fertilizer N and addition of organic crop residue under CA for three years might form protein-like organic N compounds in soils (Li et al. 2019). This was evident by an increase (~10 to 14%) in potentially mineralizable (hydrolyzable) organic N fractions viz., amino acid, amino sugar and hydrolyzable ammonium in soils with CA as compared to CT (Supplementary information Fig. S5). Among the

methods, SB extracted the highest amount of N followed by $PB > CC > PP$ due to its (SB) unique extraction mechanisms: firstly, reactions of bicarbonate ions with ions/compounds, which physically protect the protein-like compounds for release of organic N (MacLean 1964) and secondly, alkaline solubilization of hydrolyzable organic N including amino acids and hydrolyzable proteins (Michrina et al. 1982). Next to SB, PB could extract N efficiently from the potentially mineralizable organic N fractions of organic-rich CA soils, because phosphate ions react with physically protected organic N compounds (Matsumoto and Ae 2004; Mukherjee et al. 2021b) and result in a higher value of extractable N. A lower extraction of PP might be ascribed to its inefficiency in extraction of soil N, since it failed to extract protein-like N compounds bound to lignin and tannin or protected by metal-organic matter complexes from soils under CA (da Silva et al. 2019; Mukherjee et al. 2021a; Saha and Mandal 2011; Stockdale et al. 2002). It (PP) could only extract easily oxidizable organic N compounds like amino acids and amino sugars (Sahrawat and Burford 1982). Due to lack of strong N extraction

Table 5 Ranking of the methods for estimation of available N in soils based on the suitability criteria for different cropping systems under conservation agriculture

Methods	Criteria 1	Criteria 2	Criteria 3	Criteria 4	Criteria 5	Mean score	Rank
Rice-maize-cowpea							
Phosphate buffer	1	1	2	1	1	1.2	1
Calcium chloride	3	4	4	4	2	3.4	4
Sodium bicarbonate	4	3	3	3	3	3.2	3
Potassium permanganate	2	2	1	2	2	1.8	2
Rice-mustard-black gram							
Phosphate buffer	1	1	2	1	2	1.2	1
Calcium chloride	3	4	3	3	3	3.2	3
Sodium bicarbonate	3	3	2	4	4	3.2	3
Potassium permanganate	2	2	1	2	1	1.6	2
Rice-cauliflower-rice							
Phosphate buffer	1	1	2	1	1	1.2	1
Calcium chloride	2	3	4	3	3	3.0	3
Sodium bicarbonate	3	4	3	2	4	3.2	4
Potassium permanganate	1	2	1	1	2	1.4	2
Across different cropping systems							
Methods	Mean score			Final mean score	Final rank		
	Rice-maize-cowpea	Rice-mustard-black gram	Rice-cauliflower-rice				
Phosphate buffer	1.2	1.2	1.2	1.2	1		
Calcium chloride	3.4	3.2	3.0	3.2	3		
Sodium bicarbonate	3.2	3.2	3.2	3.2	3		
Potassium permanganate	1.8	1.6	1.4	1.6	2		

Criteria 1, relationships with plant parameters; Criteria 2, relationships with mineralizable N fractions; Criteria 3, relationships with inorganic N; Criteria 4, responsiveness to soil total N; Criteria 5, magnitude of variability (CV%) and sensitivity i.e., maximum/minimum with each method

mechanism involving ion exchange, chelation or solubilization, PP extracted a lower amount of N than SB or PB (Mukherjee et al. 2021a, b). On the other hand, CC also extracted N from the PMN fractions following a similar extraction mechanism like SB or PB. However, CC recorded a lower value than the others (Fig. 1), as chloride has a lower affinity than phosphate and bicarbonate ions for disruption of the physical barrier created by metal-organic matter complexes and a lower capacity than PP to release easily oxidizable organic N in the solution (Michrina et al. 1982).

Nitrogen availability in soils under CA

Higher N availability in soils with ZT or RT in RMaC and RMuB systems was in line with the observations of many researchers, who reported that ZT increased available N content in soils over CT in different cropping systems and climatic conditions (Jat et al. 2018; Lv et al. 2023; Nandan et al. 2019; Saurabh et al. 2021; Wu et al. 2021). ZT or RT involved minimum disturbance in soils, particularly in the surface layers; therefore, minimized loss of soil organic matter (Ghosh et al. 2023) and subsequently resulted in

higher total and available N content (Mattila et al. 2023; Page et al. 2020; Parihar et al. 2018; Yadvinder-Singh et al. 2015). This may primarily be due to the physical protection of soil organic matter in aggregates with ZT (Ghosh et al. 2023; Nandan et al. 2019), which maintained and/or increased N content by reducing their loss through decomposition and erosion and by sequestering N in soils (Nandan et al. 2019; Page et al. 2020). Repeated tillage to soil under CT, particularly at the surface layer with either residue removal or incorporation accounted for higher available N during the initial years (Topa et al. 2021). Loss of excess N to the environment after plant removal in subsequent years resulted in lower N availability in soils under CT (Verhulst et al. 2013). By contrast, under the RCR system, RT had a higher value of available N than CT or ZT. Exclusion of legumes from the cropping system might be the reason for such anomaly. Integration of legumes in crop rotation not only offers a diverse diet to soil microorganisms, but also explores different soil layers for nutrient acquisition that have been leached to deeper layers, apart from fixing atmospheric N into soil (Hazra et al. 2018; Page et al. 2020). These helped RMaC and RMuB systems to create a favorable micro-environment for supplying higher amounts of available N with any degree of conservation-tillage (ZT or RT) than that with CT.

While comparing the effect of residue + nutrient management, the treatments comprising of 100% crop residue may be subjected to short-term immobilization of N, especially in the initial years and subsequently reduced N availability in surface soils (Singh et al. 2021; Topa et al. 2021; Yadvinder-Singh et al. 2015). Three years of CA practices with 50%NPK probably was not sufficient to reverse this depletion in soil N availability, so, 100R + 50NPK had the lowest available N. Treatment receiving only 100%NPK without crop residue resulted in a greater available N immediately after its application and could not sequester the extra available N in soil system after crop removal (Page et al. 2020; Sithole and Magwaza 2019). In the case of 50R + 100NPK, amount of residue was not high enough to hamper N availability in soils due to immobilization; but could help to minimize the loss of excess available N present in soil after plant uptake. Subsequent N mineralization of organic crop residue and recommended N fertilizers helped achieve the highest N-availability among the

residue + nutrient treatments for all the cropping systems as estimated by PB, SB, CC, and PP methods.

Distribution of soil organic matter and nutrients in soils under CA differs from that under CT as tillage, residue management and crop rotation increase the storage of nutrients and their availability at surface soil (Jat et al. 2018; Nandan et al. 2019; Saurabh et al. 2021; Ye et al. 2019). Coupling with a greater input of nutrients through crop residue plus recommended fertilizers under diversified crop rotation, CA could increase N availability in soils relative to CT. Although N may be immobilized in soil, the other losses of N viz., leaching, runoff and volatilization loss from soils are restricted considerably under CA (Michael et al. 2021; Nayak et al. 2022). These helped ZT + 50R + 100NPK or ZT + 100R + 75NPK to retain the highest available N among the CA treatments, whereas CT + 100R + 50NPK retained the lowest. Jat et al. (2018) reported a similar increase in available N content in soils (33 and 68%, respectively) under CA-based rice-wheat-maize and maize-wheat-mung bean cropping systems over the conventional agricultural practices after four years in reclaimed sodic soil of north-west India. Similar increase in N availability in soils with attendant increase in crop yield and N use efficiency under CA (Supplementary Information Figs. S3 and S4) was also reported by others from the sub-tropical Brahmaputra Floodplain agroecological zone (Kader et al. 2022).

Selection of suitable method(s) for estimation of available N in soils under CA

Relationships of available N in soils estimated by different methods with crop parameters may be linked to their chemical composition and nature of the extracted compounds (Matsumoto et al. 2000a, b; Mukherjee et al. 2021b). Matsumoto and Ae (2004); Matsumoto et al. (2000b) reported that PB extractable organic N (PEON) compounds are less polymerized, easily mineralizable and protein-like in nature. PB extractable N also accounts for PMN, which contributes a major share to plant-available N fractions in soils rich in organic matter (da Silva et al. 2019; Matsumoto et al. 2000b). Further, Higuchi (1982) and Senwo and Tabatabai (1998) demonstrated that PEON compounds can maintain a uniform and low C: N ratio (12:1 to 14:1) regardless of the soil types and nutrient management practices. All these indicated

that N mineralization and subsequent N availability from protein-like PEON compounds could maintain crop needs for N (Mukherjee et al. 2021a). On the other hand, unlike PB, SB and CC extractable N compounds might have different amino acid compositions and molecular sizes as observed from the discrete and higher values of C: N ratio (Matsumoto and Ae 2004; Matsumoto et al. 2000a, b; Mukherjee et al. 2021b), which might govern a mismatch between N availability and actual crop need. Because of these, PB excelled over SB and CC in establishing significant relationships with all the plant parameters under CA with different cropping systems (Table 3). Besides, PP mostly measures the reactive N species of high mobility, while ignoring the most labile PMN fractions (Saha and Mandal 2011; Stockdale et al. 2002). As it is a weak oxidizing agent, PP is inefficient in extracting protected protein-like compounds, which are complexed with other refractive fractions of organic N (Stockdale et al. 2002) and contribute to the yield of available N in soil (da Silva et al. 2019; Liu et al. 2016). After three years of CA with inorganic and organic (residue) N addition, the system may not be mature enough to retain N in more complex organic forms, which generally happens in long-term organic-rich systems like CA or organic farming. This short-term CA could not restrict PP to measure the easily mineralizable organic N fractions present in soils and the difficulties related to N extraction from PMN fractions did not appear in this study. As a result, PP had strong relationships with grain and stalk N, its uptake by crop and grain yield. Even in some cases, the relations were at par with PB in addressing plant parameters.

Better relationships between PB extractable N with the most contributory hydrolyzable organic N fractions further indicated the superiority of PB over the other methods (Table 4). Although PP established strong relationships with inorganic N for all the cropping systems over the other extractants, these relationships could not improve the correlations with plant parameters over PB. A lower contribution of inorganic N to total N (~4–7%) may be the reason behind this, while PMN fractions, mostly represented by the other methods, may contribute to around 40% of total N in organic-rich systems (da Silva et al. 2019; Mukherjee et al. 2021b). CA facilitated a greater total N sequestration in soils in organic forms due to addition of organic N through rice residue for the last

three years. Possibly, PB could extract these organic N fractions more efficiently than the other methods and therefore, was more responsive to total N in soils (Fig. 3). In the case of coefficient of variation and sensitivity analysis, the higher values are preferred as the soils under CA are subjected to perturbations and management practices. PB and PP accomplished the higher values (Fig. 4) and established their advantage over the other methods for all the cropping systems. The results of PCA further established the potential of PB among the methods of available N extraction (Supplementary information Table S2 and Fig. 5). The highest score of PB in the first principal component indicated its significant influence on crop performance over the other methods. PCA-biplot also depicted the closest relation of PB followed by PP with crop parameters and easily mineralizable N fractions. In contrast, SB and CC could not relate well to the same. Overall, PB was proved to be the best method for assessing the N availability in CA soils followed by PP.

Conclusion

On average, CA practices beyond three years significantly improved N availability in soils of the lower Indo-Gangetic Plains. Of the tested 15 practices, ZT with 50–100% rice residue retention plus 75–100% N, P and K fertilizer for legume-based RMaC and RMuB cropping systems, and RT with 50% rice residue plus 100% N, P and K fertilizers for cereal-based RCR system ensured adequate N supply in soil for nutrition of crops. Selection of these practices and cropping systems would overcome the problems of N nutrition of crops under CA. A novel multi-criteria-based technique identified PB as the best method for estimating available N in soil under CA with rice-based cropping systems, particularly, in the initial years. This study calls for validation of PB method to assess plant available N in other soil types and crop rotations under long-term CA practices.

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Data availability Raw data that support the results of this study are available from the first and corresponding authors upon reasonable request.

Declarations

Competing interests The authors have no known competing financial or non-financial interests to disclose.

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