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Research Article

Biological Nitrogen Fixation by Local and Improved Genotypes of Cowpea in Burkina Faso (West Africa): Total Nitrogen Accumulated can be used for Quick Estimation

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Biological nitrogen fixation (BNF) by legumes is an indicator of their potential contribution to recycling nitrogen in cropping systems. Many techniques exist for the quantitative measurement of legume BNF. The isotopic dilution (ID) methods are the most accurate but are too expensive, time-consuming and require technical expertise. There is a gap between the simple but less accurate Total Nitrogen Difference (TND) method and the Isotopic Dilution (ID) methods. By measuring the BNF of 11 cowpea (*Vigna unguiculata*) genotypes, this study aimed to develop a simple model as an improved tool for the quick estimation of BNF. Total N accumulated by traditional genotypes from Burkina Faso varied from 23 to 41 kg ha^{-1}. Approximately 40 to 65% of this was nitrogen derived from the atmosphere (Ndfa) when the TND method was used (Ndfa-TND), while the ID method indicated that 29 to 37% of N accumulated was derived from the atmosphere (Ndfa-ID). The TND method overestimated the BNF of high N-yielding genotypes but underestimated the BNF of low N-yielding genotypes (N-accumulated below 31 kg N ha^{-1}). The relationship between N-accumulated and Ndfa-ID was described by a polynomial regression: $Y_i = 0.0127\ X_i^2 - 0.5354\ X_i + 17.44$, where Y_i and X_i represent Ndfa-ID and N-accumulated, respectively ($P<0.05$, $R^2 = 0.92$). The model was validated and could be used for quick estimation of BNF directly from the N accumulated.

1. Introduction

It is estimated that more than 70% of African soils are inadequately fertile or degraded by agricultural practices and human and animal pressure [1, 2]. At the same time, the weak revenues of small farmers limit the application of nutrient inputs such as chemical fertilizers. Biological nitrogen fixation (BNF) by legumes contribute to recycling N in cropping systems. They perform well in intercropping systems, which are very important in developing countries, as well as in low-input and low-yield farming systems [3–6]. The cropping systems of small farmers comprise many nitrogen-fixing legume crops, such as groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* (L.) Walp.), which are usually rotated or intercropped with cereals. In the complex and multicropping systems of small farmers, the fodder and residues of legumes constitute an important source of animal feed, but the remainder of the fallen senescent leaves and underground parts are sources of organic matter of good quality that improve soil mineral N, increasing not only the yields of the succeeding cereal crop but also its nitrogen use efficiency. For example, Bado et al. [3] showed that groundnut and cowpea could increase soil mineral N by 36 to 52% and the yields of succeeding sorghum by 50 to 300% as a consequence of an increase of both soil and fertilizer N use efficiency and other rotational benefits like pests, disease, and weeds. At farmer's levels, both grain and biomass yields are the main criteria for the adoption of legume genotypes, meaning that the total N accumulated in the aboveground part is a useful indicator for recommendation and adoption

of legume genotypes by farmers. For researchers, a quick estimation of BNF could help screen for legume genotypes as a first step before testing at farmer's fields.

Many methods have been developed for the measurement of legume BNF. Except for the oldest and simplest method of total N difference [7], most of the methods are expensive, time-consuming, and difficult to implement at the farmer's level [8]. The oldest method of total N difference (Ndfa-TND) calculates the N difference of N accumulated in N_2-fixing and nonfixing plants (control). The TND method has many comparative advantages: simplicity, rapidity, affordability, and ease of implementation without great expertise. However, this method is less accurate than the methods of isotopic dilution (ID). In general, the TND method overestimates BNF compared with isotopic methods [7, 9]. While making more accurate estimations of BNF, ID methods are also the most expensive and time-consuming. Except the natural abundance method, ID methods require the application of enriched ^{15}N fertilizers, technical expertise, and highly technical laboratory equipment for ^{15}N analysis [7, 10, 11]. The ID methods are not really appropriate for the day-to-day estimation of BNF. From the simplest, easiest, and most affordable but less accurate method of TND to the most accurate isotopic method, there is a gap of tools for a quick estimation of BNF.

Crop models have recently been developed to simulate the growth and nitrogen fixation of legumes [12]. For example, the CROPGRO model [13] simulates crop development in daily time steps with many requirements of input data of weather, soil parameters, and others to calculate N_2 fixation [12]. In a review of published literature of the approaches used to simulate, rather than measure, legume BNF, Yanyan et al. [8] showed that most simulation models estimate the N fixation rate from a predefined potential N fixation rate, adjusted by the response functions of soil temperature, soil/plant water status, soil/plant N concentration, plant carbon (C) supply, and crop growth stage. All these models require detailed information on the environment and plant genetic performance in addition to historical crop datasets for calibration and validation.

It is communally observed that total N accumulated by N2-fixing legumes seems to be proportional to N derived from atmosphere. Otherwise, high level of total N accumulated is an indicator of the high capacity for BNF [3]. In this research, we postulated that the relationship between the two factors (N accumulation and N derived from the atmosphere measured by the ID method) could be used to develop a model equation for quick estimation of BNF.

2. Materials and Methods

2.1. Experimental Site and Material. The study was conducted in Burkina Faso (West Africa). The climate of West Africa is governed by the Inter-Tropical Convergence Zone (ITCZ), causing moist conditions in the north through a unimodal rainy season (May-October), and the "northeast trade" wind from north to south (October-April), a cold and dusty wind bringing rainfall to the south [14]. The climate of Burkina Faso is characterized by a dry season, which lasts, on average, from mid-November to mid-April, and a wet season, which lasts from May to mid-September. Average temperatures vary from 25°C in January to 32°C in April with a night-day variation of 20°C. Temperatures can oscillate strongly from 15°C during the night to more than 40°C during the day. The relative humidity varies between 6% during the dry season and 95% in the rainy season [14, 15]. The experiment was carried out at the National Agricultural Research Institute (INERA) at the research station of Farakô-Ba (4°20′ West, 11°6′ North and 405 m altitude above sea level). In general, planting dates occur in June and harvesting in October. The total rainfall of the year of the experiment (2012) was 1071 mm and could be considered a normal season (Figure 1). The soil used in the experiment was an Ultisol, a weakly acidic sandy soil with low clay and organic C contents. Available P (P-Bray I), Ca, Mg, and exchangeable K were very low (Table 1). Three traditional and eight improved genotypes were used (Table 2). A nonfixing genotype of cowpea (IC-1) was used as control or test plant [16]. The recommended N-P-K mineral fertilizer supplying 14 kg N ha^{-1}, 10 kg P ha-1, and 11 kg K ha^{-1} was used.

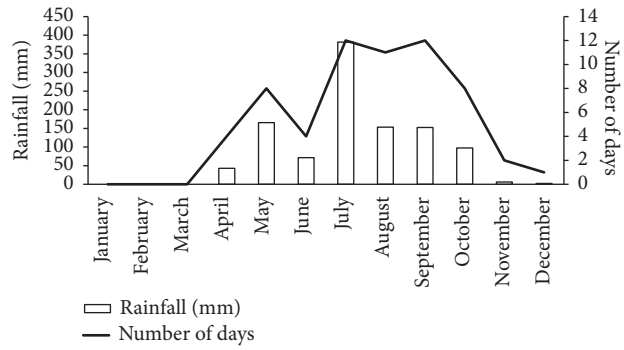

Figure 1: Number of days of rain and monthly rainfall during the experimental year 2012.

2.2. Experimental Design and Treatments. The soil of the experiment remained under fallow for four years. To minimize the variation in fertility before the experiment, the soil was cropped to sorghum for one season. Half the application rate (30 kg ha⁻1 of N, 8 kg ha^{-1} of P, and 6 kg ha^{-1} of K) of recommended fertilizer for sorghum was used with N-P-K-S-B mineral fertilizer. After this first cropping season of sorghum, the experiment was laid down in the second season with cowpea genotypes. At the start of the experiment, soil samples were taken from the top 20 cm depth for laboratory analyses. Soil pH, exchangeable acidity [17], organic carbon [18], and nitrogen were determined. The experimental design was a randomized Fisher block with 12 treatments corresponding to 12 genotypes (11 N_2-fixing and 1 non-fixing genotype). The nonfixing cowpea was utilized as a control or test plant to calculate the nitrogen derived from the atmosphere with the total nitrogen difference method [7]. The 12 genotypes were sown at densities of 125,000 plants ha^{-1} on 40 cm × 40 cm spacing, 2 plants per hill. Each experimental plot

Table 1: Main physicochemical characteristics of the topsoil (0-20 Cm).

Clay (%)	6		
Sand (%)	72	pH H_2O	6.1
Silt (%)	20	pH KCl	5.2
Organic C (%)	0.59	Ca^{++} ($cmol^{+}$ kg^{-1} soil)	1.10
Total N (mg kg^{-1})	410	K^{+} ($cmol^{+}$ kg^{-1} soil)	0.05
Total P (mg kg^{-1})	79.0	Mg^{++} ($cmol^{+}$ kg^{-1} soil)	0.40
Avail.-P Bray I (mg kg^{-1})	5.0	ECEC (cmol+ kg^{-1} soil)	1.70
Available K (mg kg^{-1})	543		

Table 2: Grain, shoot, and total biomass yields of local and improved genotypes and nonfixing cowpea.

Types and names of cowpea genotypes		Shoot, grain and total biomass yields (kg ha^{-1})		
Cowpea genotypes	Names	Shoot	Grain	Biomass
Local genotypes	Gorom local	433	313	746
	Moussa local	477	419	896
	Boussé local	759	741	1500
Improved genotypes	11K	1690	515	2205
	11P	2080	533	2613
	Bambey -21	800	633	1433
	IAR 7/180-4-5-1	1338	647	1985
	KVX 414-22-72	785	678	1463
	IT 89KD374-57	602	771	1373
	KN 1	952	800	1752
	TVX -3236	1047	901	1948
Non-fixing genotype	IC-1	301	264	566
Standard Error		101	49	146

measured 24 m^2 (6 m × 4 m). Ten kg ha^{-1} of P with Triple Super Phosphate and 11 kg ha^{-1} of K with potassium chloride were applied 10 days before sowing on all plots. Five days after sowing, nitrogen fertilizer was applied at 14 kg N ha^{-1} in the form of urea. All plots were maintained weed-free by manual weeding. Cowpea genotypes were not inoculated and were colonized only by native rhizobia preexisting in the soil. The grain and shoots of cowpea were harvested at maturity, leaving two border rows to eliminate edge effects. Total weights of shoots and grains were measured after 45 days of air-drying after harvest.

2.3. Assessment of Biological Nitrogen Fixation. In the same plots of the agronomic experiment, microplots of 3.2 m2 (1.6 m × 2 m) were laid down in the main plots. To avoid runoff and ensure equal access to ^{15}N, microplots were delimited by metallic sheets. The same uniform dose of 10 kg ha^{-1} of P and 11 kg ha^{-1} of K was applied 10 days before sowing in the microplots as for the main plots. For the isotopic dilution method (ID), small dose of 14 kg N ha^{-1} by ammonium sulfate with 10 at.% ^{15}N excess [10] was applied at the same dose in the microplots five days after sowing. To ensure a uniform distribution of the applied ^{15}N, the ammonium sulfate was diluted in water before application. Microplots were maintained weed-free during the cropping by manual weeding. Cowpea genotypes were not inoculated and were colonized only by native rhizobia preexisting in the soil. We checked through visual observations that fixing nodules were not observed on the roots of nonfixing genotypes while active nodules colonized the roots of N-fixing genotypes.

The shoots of 12 plants (0.96 m^2) from the microplots were harvested at flowering, leaving one border row to eliminate edge effects. Plant samples were dried at 60°C for 72 hours and ground for laboratory analyses. An elemental analyzer coupled to an isotopic ratio mass spectrometry at the IAEA Seibersdorf Laboratory was used to determine total N in shoot samples and atomic percent 15N excess.

Nitrogen fixed by cowpea genotypes from the atmosphere was calculated using two methods: total nitrogen difference (TND) and isotopic dilution (ID). Using the TND method, nitrogen derived from the atmosphere was calculated by subtracting the total nitrogen of the nonfixing cowpea from the total nitrogen accumulated by the nitrogen-fixing cowpea genotype [7].

$$\begin{aligned}\text{Ndfa\_TND (kg ha} - 1) &= \text{N\_acc\_Fixing Cowpea (kg ha} - 1) \\ &\quad - \text{N\_acc\_Non Fixing Cowpea (kg ha} - 1)\end{aligned} \tag{1}$$

where N_acc is the total nitrogen accumulated and Ndfa_TND is the nitrogen derived from the atmosphere with the total nitrogen difference method.

The ID method utilizes the excess of ^{15}N of the N_2-fixing and the nonfixing cowpea to assess BNF [10]. Nitrogen derived from the atmosphere was calculated using the percentages of ^{15}N excess in N2-fixing cowpea and the nonfixing cowpea (IC-1) [16] as reference test crop (see (2)) [10]. The nonfixing cowpea filled the essential criterion as a test crop for the application of isotopic dilution method [10].

$$\text{Ndfa} - \text{ID}\ (\text{kg ha} - 1) = \left(1 - \frac{\text{A}}{B}\right) \times C \qquad (2)$$

where A= ^{15}N a.e N-fixing legume, B= ^{15}N a.e N-fixing legume, and C= Total N (kg ha^{-1}) Non-fixing legume. % ^{15}N a.e represents the percentage of atom excess ^{15}N (in the N_2-fixing and nonfixing plant) and Ndfa-ID is the nitrogen derived from the atmosphere calculated by the isotopic dilution method.

2.4. Data Analysis. The statistical analyses of agronomic data, N yields, and BNF were subjected to analysis of variance (ANOVA) [19] using Genstat Discovery Edition 3. The relationships between nitrogen derived from the atmosphere obtained by the two methods were tested with different models. The adjusted R^2 value from regression analyses, the F-test, and the root mean square error (RMSE) from statistical analyses were compared. The models that better described the relationships between variables with the highest R^2 were selected. To validate the model, a dataset from a similar experiment conducted on the same site [3] was used. This experiment was also conducted with the same ID method with ^{15}N and the dataset was used to test the agreement between the measured data and data simulated by the model equation.

3. Results and Discussion

The soil was sandy (79%) with a low clay (6%) and organic carbon content (0.5%) and was weakly acidic (pH= 6.5). The total N (410 mg kg^{-1}) and available P (5.5 mg P kg^{-1}) were very low. The exchangeable bases Ca++, Mg++, K+, and ECEC were also very low (see Table 1).

3.1. Grain and Shoot Yields. The shoot and grain yields, N yields, and BNF by cowpea genotypes are presented in Table 2. The variations in grain and shoot, total N yields, and BNF were affected by genotype ($P<0.01$). The nonfixing cowpea genotype produced the lowest yields of grain and shoot. By using only one source of nitrogen from the soil, the lower yields of the nonfixing cowpea could be explained by the fact that nitrogen was a limiting factor compared with the N_2-fixing genotypes, which have access to two sources of nitrogen (soil and atmosphere). Compared with improved genotypes, shoot production in traditional genotypes was low (0.4 to 0.8 t ha^{-1}). Grain yields were also very low (0.3 to 0.7 t ha^{-1}). Except for one genotype (Boussé local), the remainder of the traditional genotypes had the lowest yields (see Table 1). Grain and shoot yields increased with improved genotypes. The shoot yields of the improved genotypes varied from 0.6 to 2.1 t ha^{-1}, and their grain yields varied from 0.6 to 0.9 tons ha^{-1}. Thus, the shoot yields could be increased by 260 to 525% with the adoption of improved genotypes, and the grain yields could be increased from 129 to 300%, indicating the high yield gap between traditional and improved genotypes. Among the traditional genotypes, Boussé local was the most productive and a prospective material for breeding programs. The highest shoot and grain yields were obtained with three improved genotypes (11P, 11 K, and IAR7/180-4-5). Among the improved genotypes, TVX-3236 produced the highest grain yields (0.9 t ha^{-1}), while 11P produced the highest shoot yields (2 t ha^{-1}). The three main criteria that determine whether a farmer will adopt a specific genotype are (i) more grain for food, (ii) more shoots for animal feed, and (iii) both (food and fodder) uses. For example, the traditional genotype Boussé local has both good grain and shoot yields (0.7 and 0.8 t ha^{-1}, respectively), which probably contribute to the wide dissemination of this genotype in Burkina Faso and other neighboring countries.

3.2. Biological Nitrogen Fixation. The nonfixing cowpea accumulated the lowest quantity of N (14 kg N ha^{-1}). This was an indication that, as a nonfixing legume, its nitrogen is only mobilized from the soil, which is poor in this nutrient.

Data from the isotopic analysis of two genotypes (Moussa local and Bambey 21) were accidentally lost, and the BNF was calculated with only the TND method. The N-accumulation of traditional genotypes varied from 23 to 41 kg ha^{-1}. The TND method indicated that 40 to 65% of this nitrogen was mobilized from the atmosphere, while the ID method revealed that only 29 to 37% of the nitrogen came from the atmosphere (see Table 3). As observed with agronomic yields, the improved genotypes accumulated the highest quantities of N, varying from 25 to 77 kg N ha^{1}. The TND method indicated that 43 to 82% of this nitrogen was fixed from the atmosphere, while, in the case of the ID method, the percentage of N derived from the atmosphere varied from 11 to 51%. With the isotopic dilution method, approximately 33% and 40% of N was accumulated by local and improved genotypes, respectively, from the atmosphere. Compared with local genotypes, N accumulated from the atmosphere was increased from 1 to 55% by improved genotypes. By fixing 51% of its N from the atmosphere without inoculation, 11P was the most performing genotype.

We tested different models of relationships between BNF obtained from the two methods: nitrogen derived from the atmosphere with the total nitrogen difference (Ndfa-TND) and with the isotopic dilution (Ndfa-ID) methods. Linear trends were observed between Ndfa-TND and Ndfa-ID (see Figure 2). We used a graphical method [20] to partition the data into two categories. Two perpendicular lines (vertical and horizontal) on a scatter diagram were used to separate the genotypes into two categories by maximizing the number of points in two quadrants or categories (see Figure 2). The point where the vertical line intersects with the X-axis divides the

Table 3: Total N and percentage of N accumulated from atmosphere in shoots of local and improved genotypes of cowpea using total nitrogen difference (TND) and isotopic dilution (ID) methods.

Types and names of cowpea genotypes		Total N accumulated	N derived from atmosphere (kg N ha^{-1})		N derived from atmosphere (% of total N)	
		kg N ha^{-1}	Total N Difference	Isotopic Dilution	Total N Difference	Isotopic Dilution
Local genotypes	Gorom local	23.0	8.9	8.4	38.7	37.4
	Moussa local	30.9	16.8	-	53.7	-
	Boussé local	40.6	26.5	11.6	65.3	28.6
Improved Genotypes	11K	56.9	42.8	25.4	75.2	44.6
	11P	77.3	63.2	39.4	81.8	51.0
	Bambey -21	35.5	21.4	-	60.3	-
	IAR 7/180-4-5-1	42.5	28.4	11.5	66.8	27.1
	KVX 414-22-72	26.8	12.7	8.8	47.4	32.8
	IT 89KD374-57	24.7	10.6	10.7	42.9	43.3
	KN 1	30.5	16.4	10.5	53.8	34.4
	TVX -3236	38.6	24.5	12.7	63.5	32.9
Non-fixing genotype	IC-1	14.1	-	-	-	-
Standard Error		5.8	5.8	4.2	8.8	7.3

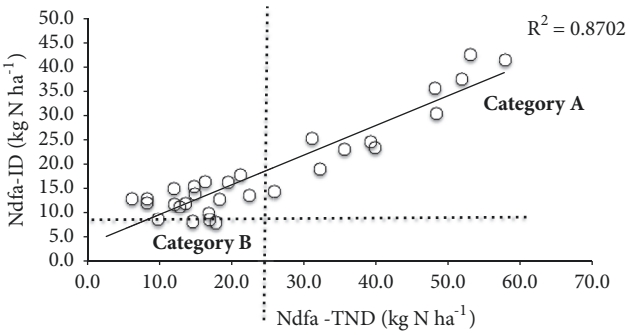

Figure 2: Relationship between Ndfa-ID and Ndfa-TND, as well as the partitioning of genotypes into two categories by utilizing a graphical method (Cate and Nelson, 1971). The two perpendicular lines (vertical and horizontal) separate the genotypes into two categories by maximizing the number of points in two quadrants or categories. By maximizing the number of points in two categories, the point where the vertical line intersects the X-axis (27 kg Ndfa-TND) divides the genotypes into categories A and B (high and low Ndfa-TND). *Ndfa-TND and Ndfa-ID: nitrogen derived from the atmosphere with total N difference and isotopic dilution methods, respectively*.

genotypes into two categories, A and B (high and low Ndfa-TND, respectively). The two categories are separated by the values of 27 kg Ndfa-TND. In the first class (A), there is no relationship between Ndfa-TND and Ndfa-ID (see Figure 2). In this class (A) (when Ndfa-TND was less than 27 kg N ha^{-1}), Ndfa-ID could not be predicted by Ndfa-TND. Otherwise, measuring BNF by the TND method cannot be used to estimate the BNF expected from the ID method. Beyond the value of 27 kg Ndfa-TND (class B), a linear relationship was found between Ndfa-TND and Ndfa-ID. This was an indication that a course estimation of Ndfa-ID could be obtained from Ndfa-TND. This linear relation could be used to estimate Ndfa-ID from the data of Ndfa-TND.

In the same way, we identified two relationships between total N-accumulated by genotypes and BNF obtained with the two methods (see Figure 3). The relationship between N-accumulated and Ndfa-TND was linear. This is explained by the fact that Ndfa-TND is obtained by subtracting the same value (14 kg N ha^{-1}, N-accumulated of the nonfixing test plant). The ID method indicates that this relationship is linear for high N-yielding genotypes (N accumulate and BNF) but not linear for low N-yielding genotypes (see Figure 3). The relationship between N-accumulated and Ndfa-ID was described by a significant polynomial regression ($P<0.05$) (see Figure 3). The regression model was

$$Y_i = 0.0127X_i^2 - 0.5354X_i + 17.44 \; R^2 = 0.92 \qquad (3)$$

where Y_i and X_i represent Ndfa-ID and N-accumulate, respectively. The high level of the coefficient of regression ($R^2 = 0.92$) indicated that 92% of the variation of Ndfa-ID is explained by the variations of total N yields of cowpea genotypes. Otherwise, the model (see (3)) could be used to calculate Ndfa-ID with an error of 8%. Moreover, the two curves (see Figure 3) explained how the two methods estimate the BNF in the two classes (A and B) presented in Figure 2. Considering that the nonfixing genotype yielded 14 kg N ha^{-1}, the intercept that separates the two categories corresponds to 31 kg N ha^{-1} (27 + 14) of N-accumulated. This is confirmed in Figure 3 at the crossing point of the two curves (TND and ID curves). Compared with the ID method, the TND method underestimated the BNF when N-accumulated was below 31 kg N ha^{-1} but overestimated the BNF when N-accumulated was higher than this value. It is generally accepted that the TND method tends to overestimate the BNF, while ID methods give the most accurate assessment of BNF (Khan and Yoshida [9]; Ankomah [21]). These data confirm but also further clarify our understanding of the misestimation of BNF by the TND method. The general conception that the TND method overestimates BNF is not always true, particularly for low N yielding plants (less than 31 kg N ha^{-1} in this experiment). However, the BNF of high N yielding plants is overestimated by the TND method.

Extensive literature provides ample evidence that the N difference method for estimating BNF is unreliable.

Using data on groundnut and cowpea, Bado et al. [3] developed a similar model equation between N-accumulated and Ndfa-ID that better estimated BNF than the TND method. The data were obtained from a similar experiment conducted on the same site with groundnut and cowpea and different fertilizer treatments. As described in this experiment, the same ID method was used to quantify the BNF of the two legumes. Despite the combination of data from two different species (groundnut and cowpea), the model predicted the Ndfa-ID from N-accumulated with an error of 6% [3]. The present model also confirms these previous results, indicating that a good estimation of BNF could be obtained from N-accumulated by using the relationship between these two parameters. To test the validity of the present model (see (3)), we used the data from Bado et al. [3] The BNF data obtained with the ID method were used as observed data (observed Ndfa-ID). The N-accumulated data were used to calculate the predicted BNF (calculated Ndfa-ID) by the model (see (3)). The relationship between the observed and calculated BNF is presented in Figure 4. The BNF was predicted from N-accumulated by the model with an error of 7%. Otherwise, the model makes a good approximation of BNF that should be obtained by the ID method.

The TND method assumes that both N_2-fixing legumes and nonfixing legumes absorb the same amount of N from the soil, which may not be true. References [21–23] estimate that the lower precision of the TND compared with isotopic methods might be ascribed to the more variable N yield component in the estimation by this method. The polynomial relationship of the ID method makes a better description of the relationship between N accumulation and BNF. This is confirmed by the better estimation of BNF by the model (see (3)) compared with the TDN method. The proposed model is developed on cowpea genotypes in the absence of inoculation (native rhizobia). The model was validated for cowpea genotypes that fix from 8 to 40 kg N ha-1 with 27 to 51% of N derived from the atmosphere. One could

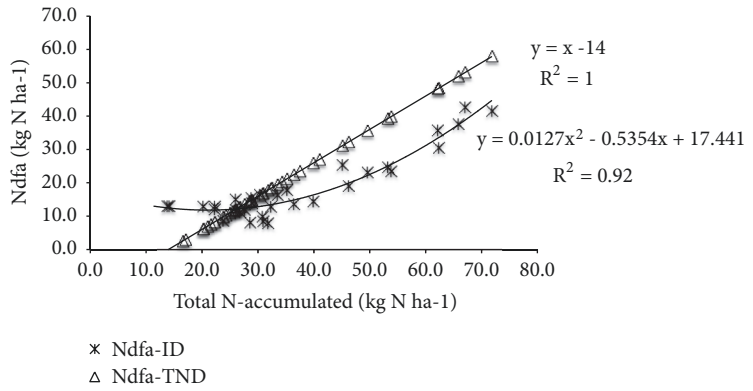

Figure 3: Relationships between total N yields of cowpea genotypes and N derived from the atmosphere as affected by the isotopic dilution (Ndfa-ID) and total N difference (Ndfa-TND) methods.

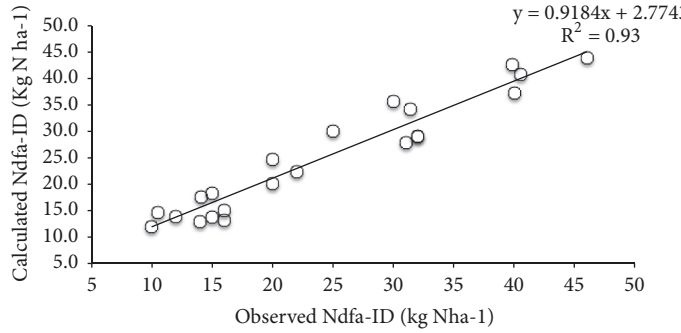

Figure 4: Relationship between the observed values of N derived from the atmosphere by cowpea genotypes cultivated with different fertilizer treatments (observed Ndfa-ID) and calculated values (calculated Ndfa-ID) from the model equation between total N yields and N derived from the atmosphere with the isotopic dilution method (Ndfa-ID). Number of values: 21; each value represents the mean of 4 values. Data from Bado et al. (2006).

argue that the model may not work with more high N-yielding genotypes, for example, in the case of inoculation with selected elite rhizobia. Being a polynomial equation, the relationship between the two factors (N-accumulated and BNF) is almost linear for high N-yielding genotypes (see Figure 3). This means that the model should work with more high N-yielding inoculated genotypes. If needed, minor adjustment of the coefficients of the model could be necessary to improve the estimation of BNF.

4. Conclusions

Good estimation of biological nitrogen fixation (BNF) by cowpea genotypes can be obtained from N-accumulated by using the proposed model: $Y = 0.0127X^2 - 0.5354X + 17.441$, where Y and X represent N dfa-ID (kg N ha^{-1}) and total N-accumulated (kg N ha^{-1}), respectively. Considering that N-accumulated is usually determined as one of the routine parameters, a quick estimation of BNF could be calculated directly from the N-accumulated by the proposed model without extra work. This simple tool could be useful for researchers, extension agents, and farmers. This method is a simple and less expensive tool for quick estimation of BNF. For example, scientists could use it for field screening of legume for BNF. Extension agents and farmers could use this model for quick estimation of BNF, as useful information that contributes for recommendation of legume genotypes. The model could be improved, adapted with more data, and validated for other legumes species.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The work was conducted as part of the FAO/IAEA Coordinated Research Project on Tropical Acid Soils.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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