$\delta^{15}N$ Values of Sorghum Grains Harvested on a Vertisol in the Semi-Arid Tropics Were Positively Related to Doses of Fertilizer N but Negatively with the Frequency of Legume Cultivation

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The relation between $\delta^{15}N$ values of sorghum grains grown on Alfisol or Vertisol fields and the fields' cropping and N-fertilizer histories was investigated. Fields were cultivated with legumes or cereals at different frequencies for 10 y, prior to sowing of sorghum. During that period, the fields for study were amended with different N-fertilizers at rates ranging between 236 and 1,472 kg N ha^{-1}. The $\delta^{15}N$ values were markedly different between the two soil types. In the Vertisol fields, the grain $\delta^{15}N$ values were positively correlated with the cereal-cropping frequency and amounts of applied fertilizers, but negatively correlated with the legume-cropping frequency. Such correlations were not observed in the Alfisol fields. It was concluded that only the amount of applied fertilizer influenced the $\delta^{15}N$ values of sorghum grains, and that this effect was caused by the slight alkalinity of the Vertisol.

Key Words: **Alfisol, fertilizer dose, legume cultivation, $\delta^{15}N$, Vertisol.**

Continuous input of nitrogen in fields is important for sustainable agriculture, because nitrogen is easily lost from the soils as gaseous N or by NO_3^- leaching. N is also removed from fields with harvested biomass. Two major N-inputs into a field include the application of chemical fertilizers and biological fixation of atmospheric nitrogen. Some of the N from these sources, which is not used by the present crop, remains in the soil and can be used by future crops. The possible origin and fate of such N-pools can presumably be studied adequately by measuring ^{15}N signatures of various soil and plant components (Yoneyama 1996).

Because they are derived from atmospheric N_2, the natural ^{15}N abundance ($\delta^{15}N$) in chemical N-fertilizers and biologically fixed atmospheric-N are close to the ^{15}N abundance in atmospheric N_2 (0‰) (Yoneyama 1996). In general, total soil N shows $\delta^{15}N$ values higher than those of chemical fertilizers or biologically fixed atmospheric-N (Yoneyama 1996). It is likely that the introduction of relatively ^{15}N-depleted N to a soil, for instance as chemical fertilizer or by biological N-fixation, may reduce the $\delta^{15}N$ value of crops growing on that soil. This was true for the application of chemical fertilizers to soils cultivated with wheat (Shearer and Legg 1975), corn (Kohl et al. 1973), and rapeseed (Karamanos and Rennie 1980). Also, continuous cultivation of an atmospheric-N_2-fixing legume (*Leucena leucocephala*) reduced the $\delta^{15}N$ values of neighbouring non-legumes (van Kessel and Roskoski 1990). However, 20 y of continuous chemical-fertilizer application did not affect corn $\delta^{15}N$ values (Meintz et al. 1975).

In view of these seemingly conflicting results, the current experiment was designed to re-evaluate the use of crop $\delta^{15}N$ values for studying the history and fate of N-pools in various soils. For this, the residual effects of various combinations of previously grown legume/non-legume crops and fertilizer applications to fields on the $\delta^{15}N$ values of sorghum, recently grown on these fields, were studied. Two soil types with contrasting pH values, Alfisol and Vertisol (Arai et al. 1998), were used, because the soil pH is known to influence soil processes that potentially result in ^{15}N-discrimination, such as ammonium-volatilization and nitrification.

Materials and methods

Five Alfisol and 10 Vertisol fields at ICRISAT (International Crops Research Institute for Semi-Arid Tropics, India) were selected and cultivated with sorghum. The pH(H_2O) and EC (electrical conductivity) values varied among the soils, probably due to the difference in the cultivation and fertilization histories (Table 1). Table 2 shows the N-contents and $\delta^{15}N$ values of different soil layers in two Alfisol and two Vertisol fields that were typical for the investigated plots. Composition and $\delta^{15}N$ values of chemical fertilizers applied to each experimental field in previous years are given in Table 3. Sorghum was sown in April 1991, without application of additional fertilizer, and grains were harvested in the autumn of that year. Every sample plot was located at a distance of between 10 and 20 m from the nearest border with neighboring fields. The grains of ten plants were combined to one sample and

Table 1. Soil pH, soil electrical conductivity (EC), frequency of cereal and legume-cropping and amount of fertilizer-N applied from 1981 to 1990 in Alfisol (R) and Vertisol (B) fields at ICRISAT.

Field designation	pH (H_2O)	EC (dS m^{-1})	Frequency of cropping seasons		Fertilizer-N applied (kg ha^{-1})
			Cereals	Legumes	
Alfisol					
RCE24 D	6.20	0.18	10 (SO, MI)[a]	3 (PP)[a]	632
RM1 A	6.50	0.15	11 (SO, MI)	3 (PP)	723
RP8 A	6.25	0.15	17 (SO, MI)	0	1,472
RP8 B	6.25	0.15	17 (SO, MI)	0	1,472
RP16 A	6.45	0.15	7 (Various)	2 (Various)	969
Vertisol					
BIL6 B	8.15	0.10	4	4 (PP, CP)	435
BM15 A	8.25	0.20	5 (SO)	5 (CP, PP)	236
BM22 A	8.10	0.22	10 (SO)	0	901
BM22 B	8.20	0.24	10 (SO)	0	901
BM22 C	8.25	0.21	10 (SO)	0	901
BP12 A	8.10	0.35	9 (SO)	1	1,006
BP14 A	8.20	0.15	6 (SO, MI)	5 (PP, GN, CP)	556
BP14 B	8.05	0.12	6 (SO, MI)	5 (PP, GN, CP)	556
BP14 C	8.05	0.10	6 (SO, MI)	5 (PP, GN, CP)	556
BS10 B	8.25	0.25	6 (SO, MI)	7 (PP, CP)	546

[a] Cereals: sorghum (SO) and millets (MI), legumes: pigeonpea (PP), chickpea (CP), and groundnut (GN).

Table 2. N content and $\delta^{15}N$ values at different depths in Alfisol and Vetrisol fields at ICRISAT.

Soil layer (cm)	Alfisol field designation	N ($mg\ g^{-1}$ DW)	$\delta^{15}N$ (‰)	Vertisol field designation	N ($mg\ g^{-1}$ DW)	$\delta^{15}N$ (‰)
0–10	RCE 4	0.42	+5.5	BR1 D	0.37	+2.7
10–20		0.49	+6.9		0.29	+1.8
20–30		0.57	+7.8		0.25	+4.7
30–40		0.59	+7.8		0.23	+4.5
40–50		0.54	+7.5		0.22	+4.6
0–10	RCW 8	0.62	+3.7	BR4 J	0.64	+2.9
10–20		0.34	+3.8		0.52	+3.9
20–30		0.21	+7.0		0.31	+3.2
30–40		0.23	+5.6		0.31	+4.8
40–50		0.21	+3.7		0.26	+4.3

Table 3. $\delta^{15}N$ values of nitrogenous fertilizers used in ICRISAT fields.

Fertilizer	N-P-K-(S) composition	$\delta^{15}N$ (‰)
Urea	46-0-0	+0.1±0.1
Diammonium phosphate (DAP)	20-20-0	−0.5±0.1
Ammonium sulfate (AS)	20.6-0-0	−0.5±0.1
Urea ammonium phosphate (UAP)	28-28-0	+2.2±0.2
Ammonium phosphate sulfate (APS)	20-20-0-(15)	+2.5±0.2

$\delta^{15}N$ data were mean±SE of three samples.

5 samples were taken per field plot (Table 1). The harvested grains were oven-dried at 70°C, ground to a fine powder, and 0.3 g of the ground grains were used for digestion and distillation (Yoneyama 1987), prior to the measurement of $\delta^{15}N$ by mass spectrometry (Finnigan Mat 251).

The natural abundance of ^{15}N ($\delta^{15}N$) in each sample was expressed as the per mille deviation from the $\delta^{15}N$ of a standard, atmospheric N_2, using the following equation;

$$\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 10^3\ (‰),$$

where $R = {}^{15}N/{}^{14}N$. The analytical precision was within 0.2‰.

Linear regression analyses of the data were performed using SPSS® 9.0 (SPSS Inc., Chicago, USA).

Results and discussion

Figure 1 shows the relationship between the $\delta^{15}N$ values of sorghum grains and the total amount of applied chemical fertilizers, cereal-cropping frequency, and legume-cropping frequency, respectively, from 1981 to 1990. For the Vertisol fields a highly significant ($p<0.001$) positive correlation was found between grain $\delta^{15}N$ values and both, amount of applied chemical fertilizer and cereal-cropping frequency, indicated by the lines in Fig. 1. For the same soils, a negative regression line was found for grain $\delta^{15}N$ against legume-cropping frequency ($p<0.04$). In contrast, grain $\delta^{15}N$ values for the Alfisol fields were not related to the amount of applied fertilizer, legume-cropping frequency, or cereal-cropping frequency (Fig. 1). The grain $\delta^{15}N$ values of sorghum grown on the Alfisol fields fluctuated around the $\delta^{15}N$ values of total soil N in the upper layers of soil (Table 2). Therefore, influences of fertilizer or cropping history on grain $\delta^{15}N$ values could easily be excluded for

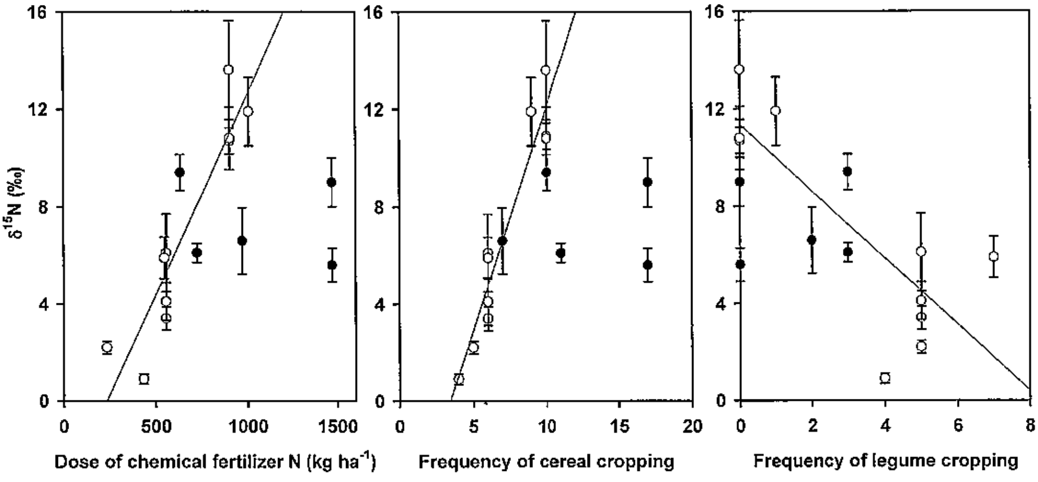

Fig. 1. Sorghum-grain $\delta^{15}N$ values (± 1 SE) in relation to the amount of applied chemical fertilizer, cereal-cropping frequency and legume-cropping frequency, during the 10-y period preceding sorghum growth. Open circles denote Vertisol fields; closed circles denotes Alfisol fields. Lines show significant correlations for Vertisols only ($n=5$).

the Alfisol fields, as could any other major isotopic discrimination during the period between the N-incorporation in the plant/soil system and final N-translocation to the grain. In accordance with observations made by Meintz et al. (1975), the $\delta^{15}N$ values of available N did not change appreciably with N-application. This was inferred from the fact that although the $\delta^{15}N$ values of the fertilizers were slightly less positive than those of the Alfisol, no difference between grain $\delta^{15}N$ values and soil $\delta^{15}N$ values was found in this soil.

Because there were no major differences in the metabolism between sorghum plants from the contrasting soil types, ^{15}N-discrimination between the time of uptake and incorporation into the grain by plants grown on the Vertisol could be excluded, in the same ways for the plants grown on the Alfisol. All three significant correlations for the Vertisol presented in Fig. 1 can be explained by the fertilization history as follows. The main difference between the Alfisol and Vertisol was the pH value (Table 1), with the Alfisol being slightly acidic and the Vertisol being slightly alkaline. Chemical fertilizers left in the soil may become ^{15}N-enriched as a result of ammonia volatilization. This process is more likely to occur in alkaline rather than in acidic soils and exerts a considerable isotopic discrimination effect (Kreitler 1975), leaving ^{15}N-enriched NH_4^+ in the soil. Therefore, although in the upper layers of the Vertisol (Table 2) the $\delta^{15}N$ values were similar to those of the artificial fertilizers (Table 3), the N taken up by sorghum may very well have been ^{15}N-enriched. Because of their respective pH, the production and uptake of ^{15}N-enriched N could have taken place to a major extent in the Vertisol only, and not in the Alfisol. Natelhoffer and Fry (1988) suggested that similar increases in soil $\delta^{15}N$ values may occur as a result of decomposition of organic matter. However, the $\delta^{15}N$ values of sorghum grains did not increase but decreased with increasing legume-cropping frequency, which excludes such a mechanism for the atmospherically derived N in this study. Rather, the relations between the two cropping histories and grain $\delta^{15}N$ values reflected the relation between exogenous N inputs and grain $\delta^{15}N$ values. Cereal-cropping history was positively correlated ($p<0.001$) with the amounts of fertilizer N, just as in the case of grain $\delta^{15}N$ values. In contrast, the legume-cropping frequency was negatively correlated ($p < 0.04$) with the doses of applied fertilizer.

In conclusion, in this study, only the amount of applied fertilizer influenced the $\delta^{15}N$ values of sorghum, and this effect depended on the soil type. More specifically, it was caused by the slight alkalinity of the Vertisol. Furthermore, this study confirms how grain $\delta^{15}N$ values can help to identify important N-transforming processes in the soil, in this case ammonia volatilization, that affect the fate of soil-N. Moreover, the strong correlation between the amount of fertilizer applied and grain $\delta^{15}N$ value for the Vertisol illustrates how, for some soil types, unknown fertilizer regimes can be deduced retrospectively from grain $\delta^{15}N$ values, after establishment of some reference points. For soil types lacking such clear correlations, such as Alfisol, this approach is not possible.

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REFERENCES

Arai, S., Hatta, T., Tanaka, U., Hayamizu, K., Kigoshi, K., and Ito, O. 1998: Characterization of the organic components of an Alfisol and a Vertisol in adjacent locations in Indian semi-arid tropics using optical spectroscopy, ^{13}C NMR spectroscopy, and ^{14}C dating. *Geoderma,* **69**, 59–70

Karamanos, R.E. and Rennie, D.A. 1980: Variations in natural nitrogen-15 abundance as an aid in tracing fertilizer transformations. *Soil Sci. Soc. Am. J.*, **44**, 57–62

Kohl, D.H., Shearer, G.B., and Commoner, B. 1973: Variation of ^{15}N in corn and soil following application of fertilizer nitrogen. *Soil Sci. Soc. Am. Proc.*, **37**, 888–892

Kreitler, C.W. 1975: Determining the sources of nitrate in ground water by nitrogen isotope studies. Report of Investigations, No. 83 Bureau of Economic Geology, p. 57, The University of Texas at Austin, Texas

Meintz, V.W., Boone, L.V., and Kurtz, L.T. 1975: Natural ^{15}N abundance in soil, leaves, and grain as influenced by long term additions of fertilizer N at several rates. *J. Environ. Qual.,* **4**, 486–490

Natelhoffer, K.J. and Fry, B. 1988: Controls on natural nitrogen-15 and carbon-13 abundance in forest soil organic matter. *Soil Sci. Soc. Am. J.*, **52**, 1633–1640

Shearer, G. and Legg, J.O. 1975: Variations in the natural abundance of ^{15}N of wheat plants in relation to fertilizer nitrogen application. *Soil Sci. Soc. Am. Proc.*, **39**, 896–901

van Kessel, C. and Roskoski, J.P. 1990: Nitrogen fixation and nitrogen cycling in a pure *Leucaena leucocephala* Stand. *In* Nitrogen Fixation: Achievements and Objectives, Ed. P.M. Gresshoff, L.E. Roth, G. Stacy, and W.E. Newton, p. 675, Chapman and Hall, New York

Yoneyama, T. 1987: N_2 fixation and natural ^{15}N abundance of leguminous plants and *Azolla. Bull. Natl. Inst. Agrobiol. Resour.*, **3**, 59–87

Yoneyama, T. 1996: Characterization of natural ^{15}N abundance of soils. *In* Mass Spectrometry of Soils, Ed. T.W. Boutton and S. Yamazaki, p. 205–223, Marcel Dekker, New York