NATURAL NITROGEN-15 ABUNDANCE OF SOIL AND PLANT SAMPLES

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Nitrogen isotope analysis of total soil N and soil-derived nitrate for nine selected Chernozemic and Luvisolic Ap horizons showed mean δ_a ^{15}N values based on atmospheric abundance of 8.8. Luvisolic soils were characterized by a relatively low level of the heavier isotope. Isotope enrichment of the total N reached a maximum in the lower B horizon. Subsoil nitrate (180-cm depth) had a δ_a ^{15}N value 1/3 that of the Ap horizon. The δ_a ^{15}N of subsurface soil horizons containing residual fertilizer N were low (-5.2) compared to the surface horizon (7.0). The δ_a ^{15}N of NH_4-N in commercial fertilizers is close to that of atmospheric-N whereas the NO_3-N has higher values. The data suggest that variations in δ^{15}N abundance between horizons of the same soil, or between different soils, may be of real use in evaluating stresses which have been placed in the nitrogen cycle due to man's activities in the past. Similarly, differences in ^{15}N abundance of soil, legume and air samples may provide an integrated estimation of symbiotic nitrogen fixation under field conditions. More detailed understanding of biological and other processes which control the N isotope concentrations must be obtained before the data reported can be further interpreted.

L'analyse isotopique de l'azote total du sol et des nitrates du sol dans neuf horizons Ap chernozémiques et luvisoliques a révélé des valeurs moyennes δ_a^{15}N de 8.8 basées sur l'abondance relative de cet isotope dans l'atmosphère. Les Luvisols ont manifesté un niveau relativement faible de l'isotope lourd. La concentration d'isotope a atteint un maximum dans la couche inférieure de l'horizon B. Les nitrates du sous-sol (profondeur de 180 cm) ont donné des valeurs δ_a^{15}N équivalent au tiers de celles des horizons Ap. Dans les horizons de profondeur contenant du N résiduel provenant de la fumure, les valeurs étaient faibles (-5.2) par rapport à celles de l'horizon Ap (7.0). Les valeurs δ_a^{15}N de N ammoniacal des engrais commerciaux sont voisines de celle du N atmosphérique, celle de N nitrique étant plus forte. Ces données font ressortir l'utilité réelle des variations de la proportion de ^{15}N entre les horizons d'un même sol et entre différents sols pour évaluer les contraintes imposées au cycle de N par les activités anthropiques passées. De même, des différences dans l'abondance de ^{15}N dans des échantillons de sol, de légumineuses et d'air peuvent fournir une estimation intégrée de la fixation azotée symbiotique dans les conditions de culture au champ. Une interprétation plus en profondeur des données présentées suppose une compréhension plus détaillée des processus biologiques et autres qui commandent les proportions de l'isotope de N.

Natural variations in ^{15}N abundance of a range of nitrogen-containing substances have been established. Twenty natural substances, including forest soil, coal, peat and pitchblend had a per mil enrichment of ^{15}N compared to reference standards ranging from -13.0 to 953 (Hauck 1973).

Generally, however, the ^{15}N content of biosphere nitrogen is restricted to a range of $\pm$ 10 δ ^{15}N units.

There is good reason to assume that the variations in the natural abundance of ^{15}N can be used to obtain qualitative and perhaps quantitative information on the relationships among N cycle processes

under various environmental conditions. Whether such variations can be used for quantifying the effects of environmental changes on the nitrogen cycle both in terrestrial and marine systems over long- or short-term periods must await an understanding of the isotope fractionation accompanying the nitrogen transformations in the abiotic–biotic systems involved (Hauck 1973; Cline 1973).

A number of biologically induced isotope effects have been reported. Biological discrimination against the heavier N isotope (^{15}N) occurs during the fixation of atmospheric N_2 by bacteria (Hoering 1955; Delwiche and Steyn 1970). Nitrogen isotope fractionation has been reported to occur during denitrification (Wellman et al. 1968; Cline 1973) and during the oxidation of ammonia to nitrate (Delwiche and Steyn 1970). Certain soil organisms which preferentially utilize NH_4-N also discriminate against the heavier nitrogen isotope, with the result that the NH_4-N remaining in the media becomes enriched with respect to ^{15}N, providing, of course, assimilation is not completed. Chemical changes such as NH_3 volatilization and ion exchange show similar effects.

The various isotope effects occurring in the soil usually result in positive $\delta\,^{15}N$ values for surface horizons. This is reflected in the data reported in the literature (Cheng et al. 1964; Bremner et al. 1966; Delwiche and Steyn 1970; Kohl et al. 1971; Edwards 1973; Feigin et al. 1974a,b). However, some forested soils show negative deviations (Riga et al. 1971), and Bremner and Tabatabai (1973) have reported a mean $\delta\,^{15}N$ value of -0.2 for 16 surface soil samples.

The study reported in this paper was carried out to determine ^{15}N enrichment of total N and soil nitrates in selected soil profiles, fertilizers and plant samples. We documented the range of ^{15}N abundance obtained on Brown, Dark Brown, and Black Chernozemic grassland soils, and also the Luvisolic-forested soils. Limited information is presented on changes in $\delta\,^{15}N$ down to depths of 270 cm.

MATERIALS AND METHODS

Soils

With the exception of profile samples from an irrigated field fertilizer experiment and a few from land seeded to tame grass, soil samples were from summer-fallowed fields which had never received nitrogen fertilizer.

Plant and soil samples, taken from a field plot experiment on a Chernozemic Black soil, were assayed for ^{15}N abundance. The barley (*Hordeum vulgare*) and faba bean (*Vicia faba*) grain and straw, and a representative sample of the surface soil, were dried at room temperature and ground to pass a 2-mm sieve.

^{15}N Assay

Analytical and ^{15}N assay procedures used are described by Rennie and Paul (1971). The analysis used for total N, including nitrate and nitrite, is a modified semimicro version of the method of Bremner (1965). The total N assay involved a pretreatment with acidified permanganate to oxidize nitrite to nitrate, followed by reduced iron and sulfuric acid to reduce the nitrate to ammonium. Kjeldahl digestion was used to convert organic nitrogen to the ammonia form. The ammonia evolved by the addition of 40% sodium hydroxide was steam-distilled using a quick-fit steam distillation apparatus with 100-ml sample flasks. Cross contamination was avoided by distilling 15 ml of 95% ethanol after each steam distillation to flush the distillation head and condenser.

Nitrate extraction was carried out using a 1:5 soil:water ratio. High speed centrifugation followed by filtration through glass fiber paper was employed to separate the soil from the solution. The extract from 150 g of soil was concentrated, using a rotary evaporator, to 25 ml. Conversion of the nitrate N to ammonia followed the procedure of Bremner and Keeney (1966).

The ammonia, from either the total N or nitrate N samples, was converted to N_2 gas by oxidation with alkaline hypobromite solution using a "Y"-tube reaction vessel. The nitrogen-isotope ratio procedure is adapted to an Atlas GD150 mass spectrometer equipped with double inlet and collector system.

Calculations

The proportion of stable isotopes occurring in nature is generally expressed as a function of the ratio of the isotopes (Craig 1957; Campbell et al. 1967; Cline 1973). Hauck (1973) similarly defined δ ^{15}N as a function of $\frac{^{15}N}{^{14}N}$ ratios, i.e.

$$\delta\ ^{15}N = \frac{\frac{^{15}N}{^{14}N}\ (\text{sample}) - \frac{^{15}N}{^{14}N}\ (\text{standard})}{\frac{^{15}N}{^{14}N}\ (\text{standard})} \cdot 1000 \quad (1)$$

In contrast, where ^{15}N enriched nitrogen compounds have been used in fertilizer management and nitrogen cycling studies, the isotope concentration has customarily been reported as percent abundance of the N, or some value derived thereof (Rennie and Paul 1971). Kohl et al. (1971) defined δ ^{15}N as the per mil enrichment of $\frac{^{15}N}{^{15}N + ^{14}N}$ and compared to its enrichment in the air. Riga et al. (1971), Edwards (1973) and Bremner and Tabatabai (1973) calculated δ ^{15}N in the same manner.

The δ ^{15}N data in this manuscript have been calculated as a function of the ^{15}N abundance and to avoid confusion and clearly differentiate these data from those derived from isotope ratios ($^{15}N/^{14}N$), our data are designed as δ_a ^{15}N,

i.e.: δ_a $^{15}N =$

$$\frac{\frac{^{15}N}{^{14}N + ^{15}N}\ (\text{sample}) - \frac{^{15}N}{^{14}N + ^{15}N}\ (\text{atmosphere})}{\frac{^{15}N}{^{14}N + ^{15}N}\ (\text{atmosphere})} \cdot 1000 \quad (2)$$

Jung and Svec (1958), in measuring the ^{15}N of atmospheric N, measured a $^{14}N/^{15}N$ ratio of 272. This corrects to an abundance of 0.3663. The reagent grade $(NH_4)_2SO_4$ had an abundance of 0.3668 compared to air designated as 0.3663. The δ_a ^{15}N of the $(NH_4)_2SO_4$ therefore was 1.4. The standard deviation for 10 $(NH_4)_2SO_4$ standards run on three consecutive days was $\pm$ 0.1 δ_a ^{15}N.

RESULTS AND DISCUSSION

A Survey of Soils

The δ_a ^{15}N values for the Chernozemic and Luvisolic Ap horizons show that, on the average, identical levels of enrichment were recorded for the total soil N and NO_3-N under field conditions (Table 1). Nitrates showed much greater variability. The values are of the same magnitude as those reported for Illinois surface soils (Kohl et al. 1971; Shearer et al. 1974), but are higher than those of 16 surface soil samples reported by Bremner and Tabatabai (1973). Cheng et al. (1964), Bremner et al. (1966), and Delwiche and Steyn (1970) report a few δ_a ^{15}N values which are equivalent to the values given in Table 1.

The lowest δ_a ^{15}N values for total N were obtained on soils which had developed under forest vegetation. This is in accord with the trends reported by Riga et al. (1971), who found that the Belgian forested samples were characterized by δ_a ^{15}N in the negative range.

δ_a ^{15}N of Soil-derived NO_3-N

The δ_a ^{15}N values of the soil-derived NO_3-N (Table 1) correspond to those recorded for total N with one significant exception, the Weyburn 1 sample. Studies reported elsewhere have indicated that the ^{15}N enrichment of NO_3-N varies widely with time, and is consistently lower than that of the total soil N (Feigin et al. 1974a; Delwiche and Steyn 1970; Edwards 1973). Feigin et al. (1974b) obtained constant and presumably characteristic δ_a ^{15}N values for soil-derived NO_3-N after 5 wk of incubation. The δ_a ^{15}N values for soil-derived NO_3-N from the 21-day incubated and from field samples for two Chernozemic soils are given in Table 2. Contrary to data reported elsewhere (Delwiche and Steyn 1970; Bremner and Tabatabai 1973; Edwards 1973), the values for the NO_3-N obtained on incubation were only slightly lower than those in the field samples. This reflects two environmental factors: (1) neither soil had received significant amounts of fertilizer N in the past; (2) the field samples were taken from summer-fallow fields (biological N transformation in quasiequilibrium).

The similarity between the δ_a ^{15}N of total soil N and of NO_3-N extracted from nonincubated samples from this survey, with comparison to similar data reported elsewhere, is illustrated in Fig. 1. The data

Table 1. $\delta_a{}^{15}N$ values for Ap horizon samples of selected soils

Soil[†]	Total N		NO$_3$-N	
	μg/g	$\delta_a{}^{15}N$	μg/g	$\delta_a{}^{15}N$
Brown Chernozemic				
(Aridic Boroll)				
Sceptre hvc	2,225	6.4	–	–
Dark Brown Chernozemic				
(Typic Boroll)				
Sutherland c‡	1,990	8.7	23	11.0
Elstow cl	2,260	9.5	17	9.1
Scott l	1,810	9.6	13	8.4
Weyburn l‡	3,250	8.8	48	2.7
Asquith fl	1,370	8.2	17	7.5
Bradwell sl-1	2,110	10.2	16	12.8
Bradwell sl-2§	1,900	9.4	23	10.1
Black Chernozemic				
(Udic Boroll)				
Oxbow l	3,120	8.6	19	10.9
Grey Luvisol				
(Cryboralf)				
Waitville l-1	1,510	6.2	26	7.0
Waitville l-2‡	2,150	4.6	–	–
Mean ±SD//		8.8±1.2		8.8±2.9

†For further descriptions of the soils, *see* Canada Dep. Agric. Publ. No. 1455, 1974.
‡Past fertilizer history unknown.
§Grass breaking.
//The mean values for total N exclude those for the Sceptre and Waitville-2 samples, as comparable NO$_3$-N values are not available.

obtained by Feigin et al. (1974a,b) for samples which had been incubated for 29 wk and for a number of the samples reported by Bremner and Tabatabai (1973) fall essentially on the same regression line.

Edwards (1973) concluded that the problem of obtaining a representative soil sample for use in establishing the isotope content of soil-derived nitrate for field investigations in insurmountable, as there is no logical basis for choosing one incubation time over another to reflect the field environment. This observation is supported by the data he reported and by that of Bremner and Tabatabai (1973).

The data reported in this study afford a rather marked contrast to that from the Midwest, in that the $\delta_a{}^{15}N$ values of NO$_3$-N mineralized under field conditions not only approximates that of total soil N, but also

Table 2. Nitrate contents and $\delta_a{}^{15}N$ values for incubated vs. field samples of two Dark Brown Chernozemic soils

	Asquith fl		Elstow cl	
	N (μg/g)	$\delta_a{}^{15}N$	NO$_3$-N (μg/g)	$\delta_a{}^{15}N$
Total	1,370	7.7	2,260	9.4
Incubated†	27	7.4	25	7.8
Field Sample	17	8.3	17	9.1

†The samples were leached prior to a 21-day incubation period.
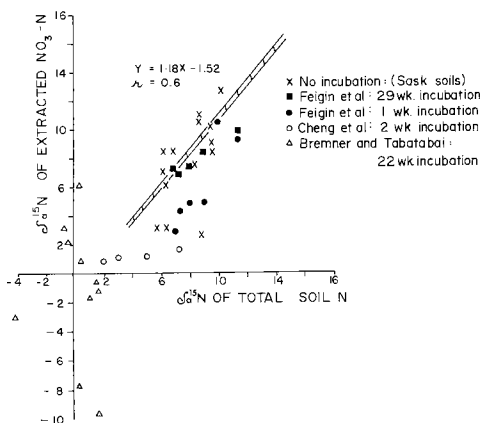
Fig. 1. The relationship between the $\delta_a\ ^{15}N$ of total soil N, and of NO_3-N extracted from nonincubated samples; data from other workers is plotted for ready comparisons only, and not reflected in the regression line.

did not differ appreciably from that produced during a 21-day incubation period. This lack of agreement is significant in view of the intensive vs. extensive soil management practices (i.e. high vs. very low fertilizer N inputs) in the Midwest and Great Plains regions, respectively.

Variations with Depth

Only slight variations were recorded in the isotopic composition of the total N from the A, B and upper C horizons of a Chernozemic profile (Table 3); the C horizon was not reached with the depth sampled for the Luvisolic. The ^{15}N abundance was generally greatest at a depth of approximately 45 cm.
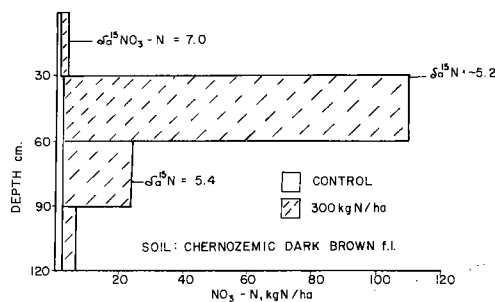

Fig. 2. NO_3-N profile (mean of replicates) on fertilized (NH_4NO_3) and control plots of an irrigated wheat experiment following harvest in September 1972. The $\delta_a\ ^{15}N$ values were measured only on one of the six replicates.

The profile recorded in Table 4 was taken to a much greater depth. The significance of the modest decrease in $\delta_a\ ^{15}N$ of total N at the 120- to 180-cm depth and the very sharp decrease in ^{15}N abundance for NO_3-N at this depth must await further investigation. The field has traditionally been in a 2-yr fallow–crop rotation, and substantial quantities of NO_3-N were found both in the rooting zone (85 kg N/ha) and below the rooting zone to a depth of 3.6 m (382 kg N/ha). The majority of this nitrogen has been derived from mineralization of soil organic matter; as far as is known, the farm field from which the samples were taken has not received any fertilizer nitrogen.

Fertilizer N

The residual NO_3-N in the 0- to 120-cm profile of two irrigated field fertilizer treatments receiving 0 and 300 kg N/ha of 34–0–0 is illustrated in Fig. 2. Substantial

Table 3. Total N and $\delta_a ^{15}N$ for two soil profiles

Depth (cm)	Chernozemic Brown hvc		Luvisolic Dark Grey l	
	Total N ($\mu g/g$)	$\delta_a ^{15}N$	Total N ($\mu g/g$)	$\delta_a ^{15}N$
0–15	2,220	6.4	2,150	4.6
15–30	1,500	6.8	1,240	4.9
30–45	1,200	7.1	830	6.0
45–60	1,170	6.4	780	6.2
60–75	990	6.1	620	5.6

Table 4. δ_a[15]N values at various depths

Depth (cm)	Chernozemic Dark Brown c			
	Total N		NO_3-N	
	μg/g	δ_a[15]N	μg/g	δ_a[15]N
0–15	1,990	8.7	23	11.0
15–120	640	6.1	22	8.5
120–150	670	6.4	22	3.1
150–180	350	5.7	23	3.1
180–240	540	6.3	31	6.2
240–270	440	6.9	45	8.5

amounts of the 300 kg N/ha rate of application were concentrated in the 30- to 60-cm depth following harvest in 1972. Of interest is the very low δ_a[15]N which characterized the nitrate N at this depth in comparison to that of the 0- to 30-cm depth.

The δ_a[15]N of the NH_4-N in a selected number of commercial fertilizers (Table 5) was consistently lower than for the NO_3-N, but in no instance were highly negative values obtained as reported by Freyer and Aly (1974); the heavier ^{15}N isotope has obviously not been discriminated against during ammonia synthesis. From the assay of fertilizer-N samples reported in this study and those reported elsewhere (Freyer and Aly 1974; Shearer et al. 1974; Hauck 1973), the percent abundance of ^{15}N in both the NH_4^+ and NO_3^- ion species can be expected to vary widely. This observation negates the possibility of relating variation in ^{15}N abundance of inorganic or organic soil-N to past fertilizer N practice.

δ_a ^{15}N of Crop Samples

Preliminary results of δ_a ^{15}N measurement on two crops, faba bean (*Vicia faba*) and barley (*Hordeum vulgare*), grown on a Chernozemic Black Loam soil (Table 6) suggest that though the differences are small in terms of absolute magnitude, it may be possible to extend field tracer methodology from its present dependence on enriched nitrogen to utilizing existing variations in natural abundance.

The ^{15}N excess of the barley samples is significantly less than that of the soil N from which the plant derived its total N requirements. The δ_a ^{15}N data for the faba bean, in contrast to the barley, reflect, in addition to discrimination against the heavier isotope during nitrification and plant uptake, the effect of symbiotic nitrogen fixation. If it is assumed that the beans derive their nitrogen from two sources, namely the air and the soil, it should be possible to apply the usual

Table 5. δ_a[15]N values for selected fertilizers

Brand	Formulation	Compound	[15]N values[†]	
			NH_4-N	NO_3-N
1	34–0–0	NH_4NO_3	−0.6	3.6
2	34–0–0	NH_4NO_3	1.0	4.8
3	21–0–0	$(NH_4)_2SO_4$	4.8	–
4	11–55–0	$NH_4H_2PO_4$	1.3	–
		Average	1.6	4.2

[†]Mean of three determinations. The mean standard deviation of measurement was $\pm$ .00031% abundance.

Table 6. Variations in ^{15}N in grain and straw of crops grown on a Chernozemic Black soil†

Crop		^{15}N excess‡	$\delta_a{}^{15}N$
Faba bean (no fertilizer)	— grain	.00056	1.5±0.5
	— straw	.00035	1.0±0.5
Faba bean (60 kg P/ha of $NH_4H_2PO_4$)	— grain	.00059	1.6±0.5
	— straw	.00024	0.7±0.2
Barley (60 kg P/ha of $NH_4H_2PO_4$)	— grain	.00153	4.2±0.5
	— straw	.00134	3.7±0.6
Soil (total N)		.00271	7.4±2.0
$NH_4H_2PO_4$		.00048	1.3±1.7

†The data are the mean of six replicates.
‡% abundance (crop) − 0.3663 (N_2 standard).

isotope dilution principle to estimate the amount of nitrogen in the faba bean that has come from the soil, i.e. .00059/.00153 × 100 ≈ 38% of the nitrogen in the bean has come from the soil, with the remainder, 62%, from symbiotic N fixation. In this calculation, the ^{15}N excess for the barley rather than the soil was utilized. This assumes equal discrimination in the uptake of ions by both crops.

CONCLUSIONS

The controversy in the literature concerning the application of natural ^{15}N abundance to fertilizer N disposition studies has, to some extent, been resolved by the paper of Meints et al. 1975. They concluded that in relation to fertilizer N studies, the method of using natural variations in abundance of ^{15}N is qualitative or, at best, roughly quantitative. The data reported in this manuscript suggest that the ^{15}N fractions associated with fertilizer N × soil reactions is complex, with both biological and physical fractionations occurring. At this time, the use of fertilizer N enriched with ^{15}N offers major advantages over dependence on natural ^{15}N variations.

There are a number of instances where normal tracer techniques using enriched ^{15}N cannot readily be applied, but where variations in natural ^{15}N abundance offer promise. Our data suggest that this includes the measurements of field N fixation integrated over a whole growing season, and the assessment of the fate of mineral nitrogen, derived from soil organic matter, which has accumulated below the rooting depth in semiarid environments such as the soils in the Great Plains Region.

An important parameter affecting the interpretations that can be made from measured natural variations in ^{15}N in any compartment of an ecosystem is the errors associated with such analyses. The mass spectrometer has adequate accuracy, as measurements of ^{15}N abundance can be made with a standard deviation of 0.2 $\delta_a{}^{15}N$. This is equivalent to 5% or less of the small differences in natural ^{15}N abundances. In our measurements of the ^{15}N abundance of plant N in faba beans and barley, a standard deviation of ± 0.5 $\delta_a{}^{15}N$ over a difference of 3 to 4 $\delta_a{}^{15}N$ was encountered. This level of error includes instrument as well as soil variability.

Many N transformations in nature have not, in the past, been measured within an order of magnitude, and normal tracer techniques using ^{15}N enrichment are often not applicable. Under such conditions, even though the use of natural ^{15}N abundance must be termed somewhat speculative, it should lead to a better understanding of the nitrogen transfer between various compartments of the nitrogen cycle, and transformation rates in natural and man-made ecosystems.

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