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SHORT COMMUNICATION

QUANTIFICATION BY DIRECT ^{15}N DILUTION OF FIXED N_2 INCORPORATION INTO SOIL BY *CAJANUS CAJAN* (PIGEON PEA)

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Significant amounts of N_2 fixed by legume crops are undoubtedly incorporated into the soil, but specifically how much? The total N_2 fixed by a particular legume includes not only the fixed N_2 in the harvested portion of the aboveground biomass, but also the fixed N_2 in the leaves and stems returned to the soil, as well as that in the roots, nodules and root exudates. Most estimates of N_2 fixation are based solely on the aboveground plant material, and the N contribution to the soil is generally ignored (Nutman, 1976; La Rue and Patterson, 1981). Both the N balance (difference) and the ^{15}N fertilizer dilution techniques employ non-fixing plants as controls. The controls are used to account for the uptake of unlabeled soil N and make analysis of soil for total N and atom% ^{15}N unnecessary (Rennie *et al.*, 1978; La Rue and Patterson, 1981; Witty, 1983). This avoidance of soil N analysis is understandable since the techniques available for soil N analysis, when coupled with spatial variability, make it difficult to measure increases in a large and variable pool of N.

The difference between the natural abundance of soil ^{15}N and atmospheric N_2 atom% ^{15}N (the delta technique) can be used to measure the incorporation of fixed N_2 into soil (Amarger *et al.*, 1979), but is fraught with difficulties, including the analytical problems of measuring small differences. Other problems include variations in soil atom% ^{15}N with depth (Steele *et al.*, 1981), and location in a field. The task is complicated further by the enrichment of ^{15}N during plant decomposition (Turner *et al.*, 1983) and plant N uptake (Kohl and Shearer, 1980). These factors combine to make it difficult using the delta technique to discern the amount of N a legume crop adds to the soil.

To quantify the amount of fixed N_2 incorporated into the soil, we used a soil which had been enriched with ^{15}N. The soil had been in lysimeters which were heavily fertilized with $(^{15}NH_4)_2SO_4$ for 2 yr between 1973 and 1975 (Focht and Stolzy, 1978). The soil was removed from the lysimeters during the summer of 1981. Over the years the ^{15}N applied had become relatively uniformly incorporated into the soil organic matter as verified when the soil was used, in a greenhouse study, to directly estimate N_2 fixation by *Cajanus cajan* (pigeon pea) via analysis of plant material for ^{15}N enrichment (La Favre and Focht, 1983a). Using ^{15}N labeled soil has several advantages over the use of ^{15}N enriched fertilizer. The ^{15}N fertilizer procedure relies on comparing the subject plant with some nonfixing control. This is essential since there are three sources of N in a labeled fertilizer N_2 fixation experiment: the fertilizer, the soil and the atmosphere. The control plant is used to correct for plant N derived from the soil. A modified isotope dilution calculation is then performed (Boddey *et al.*, 1984; Rennie, 1984; Ledgard *et al.*, 1985). With labeled soil this is unnecessary since the principles of isotope dilution can be directly applied (i.e. no control plant is necessary) to account for fixed N incorporated into the plant (Chalk *et al.*, 1983; La Favre and Focht, 1983a) as well as the soil. The analysis of the soil used by La Favre and Focht (1983a) to grow pigeon pea affords an opportunity to quantify the amount of fixed N_2 which is incorporated into the soil by direct ^{15}N dilution and is the topic of this report.

The system used to grow pigeon pea has been described by La Favre and Focht (1983a). The plants were grown in PVC columns (30 cm dia × 90 cm) in ^{15}N enriched soil (86 kg-soil per column). This soil was low in N (0.042%) and had demonstrated a very low denitrification potential (Focht and Stolzy, 1978). Columns for all treatment were amended with $CaSO_4 \cdot 2H_2O$, 0.96 g; $Ca(H_2PO_4) \cdot 2H_2O$, 2.04 g; and K_2SO_4, 4.46 g. A range of rhizobia strains with different N_2 fixation efficiencies (H_2 uptake, Schubert and Evans, 1976), were used to inoculate the plants. In addition, there were N fertilized and unfertilized controls which were not inoculated but were nodulated by inefficient indigenous soil rhizobial strains. Each treatment was replicated five times, and the plants were grown in the greenhouse for 225–252 days. At the end of the experiment the shoots were harvested, and the soil removed from each column. Coarse root material was removed and the soil was air dried and stored. Soils were sieved (<2 mm) before chemical analysis. Two samples from each column were then analyzed for total N and ^{15}N content. Total soil N was determined by Kjeldahl analysis (Bremner and Mulvaney, 1982). The ^{15}N content of the soil was determined by mass spectrometry following digestion and steam distillation (La Favre and Focht, 1983a).

The soil ^{15}N enrichment determined from stored unplanted soil samples was 0.458 atom% ^{15}N, a value which does not differ significantly (two-tailed t-test, $\alpha = 0.05$) from that determined by La Favre and Focht (1983a). The significantly reduced atom% ^{15}N values (two-tailed t-test) for the soil after the *C. cajan* final harvest indicates a substantial soil N input (Table 1). The input is due to N_2

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Table 1. Comparison of fixed N_2 incorporation into the aboveground crop (*Cajanus cajan*) and soil as directly measured by ^{15}N dilution

Rhizobium[1] strain	Soil N[2]			N_2-fixation[4]	
	atom% ^{15}N	%Nf	(mg N kg^{-1} soil)	Crop (kg ha^{-1})	Soil (kg ha^{-1})
P132	0.433 ± 0.006	27.2	423 ± 17	134	148
P148	0.429 ± 0.003	31.5	430 ± 10	115	179
HP147	0.432 ± 0.010	28.3	418 ± 12	96	153
401	0.434 ± 0.005	26.1	428 ± 14	105	144
None, −N	0.431 ± 0.012	29.3	423 ± 13	67	160
None, +N	0.423 ± 0.005	ND[3]	439 ± 16	ND[3]	ND[3]

[1]Uninoculated plants were nodulated by indigenous soil rhizobia.

[2]Atom% ^{15}N measured by mass spectrometry. Mean ± SD are given for atom% and total N determinations ($n = 5$). All atom% ^{15}N are significantly less than the enrichment before planting of 0.458 ± 0.012 atom% ^{15}N ($\alpha = 0.05$). %Nf is the percent soil N derived from fixation as defined by equations in the text.

[3]Not determined since the addition of fertilizer N (3.0 g-N plant^{-1}, as $(NH_4)_2SO_4$) precludes ^{15}N estimation of dinitrogen fixed.

[4]Assuming a plant density of 15,000 ha^{-1}. Crop values measured by direct ^{15}N dilution by La Favre and Focht (1983a).

fixation except in the fertilized treatment in which case the reduced atom% ^{15}N would be from a combination of N fertilizer and N_2 fixation.

The dilution of the originally enriched soil-N (0.458 atom% ^{15}N) by atmospheric-N (0.366 atom% ^{15}N) can be represented mathematically as (Rennie *et al.*, 1978; Hauck, 1982),

$$(X)(0.458\%) + (Y)(0.366\%) = (X + Y)(Z), \qquad (1)$$

where X is the amount of indigenous soil N, Y is the amount of soil N derived from fixation, and Z is the observed atom% ^{15}N of the soil after the *C. cajan* was harvested. The fraction of soil N derived from fixation (Nf) would then be,

$$Nf = Y/(X + Y).$$

Since this is a ratio, the absolute values of X and Y do not have to be determined to solve for Nf. We can employ an algebraic substitution of $X = 1$ in equation (1), and then solve for a corresponding value of Y, and from Y subsequently calculate the percent of soil N derived from fixation (%Nf) as follows:

$$Y = (0.458\% - Z)/(Z - 0.366\%)$$

$$\%Nf = [(Y)/(Y + 1)](100).$$

This allows the determination of %Nf solely from soil atom% ^{15}N values for soils before planting and after harvesting.

All treatments had N incorporations from fixation which amounted to at least 26% of the total soil N (Table 1). Using these figures in conjunction with the Kjeldahl determinations of total soil N, the amount of N derived from fixation and incorporated into the soil was calculated for a crop of 15,000 plants ha^{-1}. This allows comparison with the N derived from fixation incorporated into the above ground biomass (Table 1). Surprisingly, in every case the incorporation of biologically fixed N into soil was greater than the amount of fixed N incorporated into the above ground plant biomass as measured directly by ^{15}N dilution (La Favre and Focht, 1983a). Sheldrake and Narayanan (1979) found that the root systems of pigeon peas contained 10 kg N ha^{-1}. Using their estimate to account for root N still leaves the N addition to the soil greater than the N addition to the plant in every case.

The estimate of fixed N incorporated into the soil includes N from unrecovered and senescent nodules, root exudates, and fine roots. There is also the possibility that some small fraction of the N added to soil was from N_2 fixation by hydrogen-oxidizing bacteria. Hydrogen-oxidizing bacteria populations develop in the rhizosphere of low efficiency *Rhizobium* strains (i.e. 401) using the H_2 evolved from root nodules for growth (La Favre and Focht, 1983b). The H_2 oxidizer *Xanthobacter autotrophicus* is a known N_2 fixer (Aragno and Schlegel, 1981).

The value for plant biomass fixed N does not include the input from the abscissed leaves and flowers, which were not recovered in this study. In the field abscissed leaves and flowers would be incorporated into the soil. This tends to make our estimate for N incorporation into soil from N_2 fixation by pigeon pea conservative.

Acetylene reduction measurements indicate that the average total N fixation was 45% greater than could be accounted for by isotope dilution measurements of fixed N in the aboveground crop (La Favre and Focht, 1983a). Our soil analyses account for the remaining fraction of the total N_2 fixed, as measured by C_2H_2 reduction, by showing that the remainder was incorporated into the soil.

The magnitude of the soil N addition measured in this study would be virtually impossible to measure in typical agriculturel soils. This is understandable since it represents a relatively small N addition in comparison to the large soil N pool. Attempts to measure the addition in the field would be further confounded by spatial variability. We were able to measure the incorporation of N_2 fixed by pigeon pea into this soil only because of the low initial content of soil N and the high soil ^{15}N enrichment which had equilibrated over a period of years. Our results clearly demonstrate that, in this low N soil, the amount of N incorporated into the soil from N_2 fixation by pigeon pea is at least equal to the N incorporated into the aboveground plant biomass.

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