# MEASURING $N_2$ FIXATION IN LEGUMES USING $^{15}N$ NATURAL ABUNDANCE: SOME METHODOLOGICAL PROBLEMS ASSOCIATED WITH CHOICE OF REFERENCE PLANTS

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Nodulated legumes contribute significantly to the N economy of soils and, in so doing, increase agricultural yields of food crops (Dakora and Keya, 1997). The amount of N fixed in symbiotic legumes by root-nodule bacteria (species of *Rhizobium*, *Bradyrhizobium*, *Allorhizobium*, *Agrorhizobium*, *Mesorhizobium and Sinorhizobium*) has generally been assessed under field conditions using the $^{15}N$ natural-abundance technique and/or the N difference method. Although the $^{15}N$ natural-abundance technique has been used successfully for field measurements of $N_2$ fixation in cultivated food grain and pasture legumes, the method is beset by problems associated with the choice of reference plants, especially in natural settings. When using this method, the N derived from atmospheric nitrogen fixation (%Ndfa or $P_{fix}$) is usually estimated (Shearer and Kohl, 1986) as:

$$P_{fix} = \frac{\delta^{15}N_{ref} - \delta\ ^{15}N_{leg}}{\delta\ ^{15}N_{ref} - \text{B value}} \times 100$$

where $\delta^{15}N_{ref}$ is the mean $\delta^{15}N$ value of the reference plant, $\delta^{15}N_{leg}$ is the mean $\delta^{15}N$ value of the test legume, and the B value is the $\delta^{15}N$ of the legume wholly dependent on $N_2$ fixation for its N nutrition. The B value represents the value of atmospheric $N_2$ and usually incorporates the isotopic fractionation associated with $N_2$ reduction in root nodules (Shearer and Kohl, 1986).

## Suitability of Reference Plants for Quantifying Legume $N_2$ Fixation

The choice of reference plant can affect the precision of the $N_2$-fixation measurement. The reference plant is usually a non-fixing species chosen for its ability to measure the $^{15}N$ abundance of soil-N taken up by the test legume (Shearer and Kohl, 1986). Thus, the reference plant is assumed to have similar rooting pattern, root depth, and the same level of N-isotope fractionation during N uptake by the test legume. However, where the $\delta^{15}N$ of soil is independent of soil depth as in the fynbos of South Africa (Spriggs et al., 2003), the root pattern and depth need not be the same as those for the legume.

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Several studies have assessed the suitability of various plant species as references for measuring $N_2$ fixation in field legumes, using the $^{15}N$ natural-abundance method (Spriggs et al., 2003; Nyemba and Dakora, 2005). Whether in on-station or on-farm studies, intercropped cereal plants in mixed culture with legumes are generally used as the reference plants. However, below-ground transfer of biologically-fixed N by the legume to the cereal has been demonstrated for intercropped systems (Eaglesham et al., 1981) and it appears that the closer the cereal roots are to those of the legume (i.e., the more interwoven), the more fixed-N is transferred to the cereal. As shown in Table 1, the N concentration was highest in the intra-hole maize (where legume and cereal were planted in the same hole), followed by intra-row planting (where cereal and legume alternated within a row). This increase in cereal N was due to the transfer of fixed-N from the legume partner. This is evidenced by the significantly lower $\delta^{15}N$ values of the intra-hole, intra-row and inter-row maize plants relative to the mono-cultured maize, clearly indicating maize acquisition of biologically-fixed N from symbiotic cowpea. These data therefore suggest that the use of intercropped cereals (whether inter-row, intra-row, or intra-hole planted non-legumes) as reference plants is likely to underestimate $N_2$ fixation. Cereal plants from mono-cultures and/or weeds occurring outside legume stands are therefore recommended as reference plants.

Table 1. Effects of cropping system on $\delta^{15}N$ and N concentration in cowpea and maize plants grown in a mixed culture. Values followed by dissimilar letters in a column are significant at $P \leq 0.05$ (Ndakidemi and Dakora, unpublished data).

| Cropping system | Parameter | | | |
|---|---|---|---|---|
| | $\delta^{15}N$ (‰) | | N (%) | |
| | Cowpea | Maize | Cowpea | Maize |
| Sole | 2.6a | 5.3a | 1.9a | 0.9b |
| Inter-row | 2.2b | 4.7ab | 1.9a | 1.0ab |
| Intra-row | 2.0c | 4.5b | 2.0a | 1.10a |
| Intra-hole | 1.9c | 4.0b | 2.1a | 1.15a |

## Mycorrhizal Effect on $\delta^{15}N$ Value of Reference Plants

Mycorrhizal infection of plants can alter the isotopic fractionation of N during uptake by roots (Spriggs et al., 2003). Mycorrhizally-infected non-legume plants were therefore found to be unsuitable for measuring $N_2$ fixation in natural stands of *Cyclopia*. As shown in another study (Table 2), the $\delta^{15}N$ values of the reference plants were found to be highly negative and lower than those of *Cyclopia* plants in the different sites, thus, making it impossible to use those values for estimating $N_2$ fixation. To measure $N_2$ fixation in such situations would require that both the legume and reference plants are mycorrhizally infected. More research is needed on the effect of N transfer and mycorrhizal infection on the measurement of $N_2$ fixation in field legumes.

Table 2. The $\delta^{15}N$ values (means ± SE‰) of *Cyclopia* and reference plants for different study sites (Spriggs and Dakora, unpublished data).

| Site | *Cyclopia* species | $\delta^{15}N$ | | B value (‰) |
|---|---|---|---|---|
| | | *Cyclopia* | Reference plant | |
| W1 | *C. subternata* | –1.79 ± 0.19 | –5.35 ± 0.40 | –0.24 ± 0.13 |
| W3 | *C. subternata* | –0.36 ± 0.06 | –5.36 ± 1.19 | –0.85 ± 0.32 |
| W4 | *C. genistoides* | –0.11 ± 0.18 | 2.87 ± 1.24 | –0.53 ± 0.20 |
| W5 | *C. genistoides* | –0.38 ± 0.07 | –5.06 ± 1.52 | –0.86 ± 0.21 |
| W8 | *C. intermedia* | –0.63 ± 0.15 | –4.65 ± 0.48 | –1.29 ± 0.44 |
| W9 | *C. intermedia* | –0.75 ± 0.33 | –5.31 ± 1.61 | –0.54 ± 0.19 |
| W10 | *C. maculata* | –0.94 ± 0.06 | 0.48 ± 0.59 | –0.50 ± 0.20 |
| W11 | *C. maculata* | –0.84 ± 0.09 | –2.01 ± 0.52 | –0.89 ± 0.14 |
| W12 | *C. sessiliflora* | –0.00 ± 0.13 | –2.69 ± 1.09 | –0.77 ± 0.14 |
| W13 | *C. sessiliflora* | –3.64 ± 0.23 | –6.08 ± 0.34 | –0.50 ± 0.09 |

## References

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