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Use of isotopic and non-isotopic techniques to quantify below-ground nitrogen in fababean and chickpea

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Abstract

Two types of techniques (isotopic and non-isotopic) were used to quantify below-ground nitrogen (BGN) for two winter legumes, fababean (Vicia faba) and chickpea (Cicer arietinum) under glasshouse and field conditions. In the glasshouse study, estimates of BGN for fababean and chickpea, respectively, were 13 and 10% of total plant N (physical recovery), 11 and 52% (soil ^{15}N dilution), 30 and 52% (mass N balance), 39 and 53% (^{15}N shoot-labeling), 37 and 42% (adjusted ^{15}N shoot-labeling), and 33 and 43% (N balance). In the field experiment, values were 25 and 77% (^{15}N shoot-labeling), 24 and 68% (adjusted ^{15}N shoot-labeling) and 29 and 60% (^{15}N balance). When averaged across all estimates (other than physical recovery), BGN of glasshouse-grown plants represented 31% of total plant N for fababean and 48% for chickpea. By comparison, the mean values for BGN as percent of total plant N in the field study using the two methods considered likely to give the most reliable results (adjusted ^{15}N shoot labelling and ^{15}N balance) were 27% for fababean and 64% for chickpea.

Key words: Isotopic/non-isotopic techniques, nitrogen, fababean, chickpea

Introduction

Legumes play an important role in crop rotation. The impact of legumes in cereal-based cropping systems can be expressed in a number of ways. One common approach to evaluating legume contributions has been to determine net inputs of fixed N_2, calculated by subtracting nitrogen (N) removed in the grain from estimates of the amounts of N_2-fixed (Chalk, 1998; Peoples *et al.*, 1995). However, research on legume effects on soil and subsequent cereal crops has revealed an apparent paradox in that measured N benefits from legumes are often greater than might be expected from such N-balance calculations (Peoples *et al*., 1995). That the predicted effects of legumes do not coincide with the observed N benefits indicates a deficiency in our understanding of the magnitude of N inputs and subsequent cycling of N in these rotational systems. Since N-balance determinations have invariably been based on shoot-derived measures of crop N, one area that seems to require further study would be to evaluate the role that nodulated legume roots are playing in the N dynamics of cropping systems (Rochester *et al.*, 1998; Russell and Fillery, 1996b). The major inputs of below-ground N (BGN) to soil-N pools are likely to come at the end of the growing season as the plants mature and senescence.

However, contributions of legume N may also occur throughout the season as roots and nodules die or are sloughed off, and in the form of exudates and secretions (rhizodeposition). Various techniques have been used to quantify N associated with roots and nodules of legumes. The most simple and common approach has been to physically remove the roots from soil. Given the difficulty and errors associated with such an approach, considerable effort has been directed at development of ^{15}N-based methodologies. These include growing legumes in ^{15}N-enriched soil (Poth *et al.*, 1986) and *in situ* labelling of shoots with ^{15}N (Rochester *et al.*, 1998; Russell and Fillery, 1996a).

Crawford *et al.* (1997) used a sequential coring and summation technique, first proposed by Hansson and Steen (1984), in which total root production was estimated from repeated, simultaneous measurements of living roots, dead organic material and decomposition rates of dead roots and old organic material. They reported that nodulated roots of barrel medic, a pasture legume, and fababean accounted for 35 and 24% of total plant biomass, respectively, in a dry season and 29 and 20% in a wetter season. They conceded, however, that total root biomass was likely to be underestimated using the coring and summation method, although they felt it was more accurate than assessments based solely on recovery of intact root material. Values for physically-recovered roots as high as 24-31% of total plant N have been reported for green gram (Chapman and Myers, 1987), chickpea (Dalal *et al.*, 1998) and some pasture species (Bowren *et al*., 1969; Reeves, 1984), although most are <15% of total plant N.

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^{15}N methods have also been used to estimate root-derived N in soil and total BGN. Fillery and co-workers (Russell and Fillery, 1996a, b; McNeill *et al.*, 1997, 1998) confirmed the importance of the nodulated root to the legume's N economy using an *in-situ* ^{15}N shoot-labelling technique, followed by ^{15}N recovery at the end of the season in shoot and root biomass and in root-zone soil. Russell and Fillery (1996b) estimated that lupine shoots and roots contained 230 and 91 kg N ha^{-1}, respectively. Values for BGN as a percentage of the total in the four studies were lupin 28%, subterranean clover 40-42%, and serradella 37-47%, respectively. Only about 30% of the total N in the root-soil system was recovered as intact biomass. Rochester *et al.* (1998) reported BGN values of 41 and 39% for fababean and soybean, respectively. Jensen (1996) used a split-root system to label pea and barley plants with ^{15}N, then recovered the ^{15}N in plant and soil fractions in the non-labelled half of the system. He determined that 14% of the total N was associated with below-ground parts, split almost equally between intact roots and exudates. Poth *et al.* (1986) grew pigeon pea in ^{15}N-enriched soil to determine total N_2 fixed from the dilution of soil ^{15}N. At the same time, however, they determined that 58% of plant N was below-ground. The use of labelled soil was a very different approach to the more popular plant labelling and warrants further examination.

This paper reports a series of glasshouse and field experiments in which various isotopic and non-isotopic methods were used to quantify BGN of fababean (*Vicia faba*) and chickpea (*Cicer arietinum*).

Materials and Methods

Glasshouse studies

The glasshouse experiments were undertaken in a temperature-controlled (27 °C day/22 °C night), naturally lit glasshouse at CSIRO Plant Industry, Canberra (35°03'S, 147°4'E), ACT, Australia. Eight seeds of each of fababean (cv. Fiord) or chickpea (cv. Moree or Amethyst) were sown into 23-L free draining pots (28.5 cm diameter- 40 cm deep) containing either 22 kg of a 50:50 mixture of sand and soil, or river sand (in one physical recovery experiment only). The seeds of both species were treated with commercial rhizobial inoculant at sowing. Plants were supplied daily with either N-free nutrient solution or tap water. Ten days after germination, the seedlings were thinned to six per pot. Each treatment consisted of three to five replicated pots. Plants were harvested during late reproductive growth.

Field study

The field experiment was conducted at Breeza (31°11'S, 150°25'E) in the northern grain-belt of New South Wales, Australia. The soil was an alkaline (pH 7.4–8.5 in $CaCl_2$) Vertisol of heavy clay texture with a total N content (0–10 cm) of 0.175%. The experiment consisted of chickpea (cv. Amethyst) and fababean (cv. Moree) grown in large (30-10 m) plots, replicated four times. At the seedling stage, metal microplot frames, measuring 0.5-0.64 m, were placed in the ground to a depth of about 30 cm in each plot. Each microplot contained either seven (chickpea), or eight (fababean) plants. Both in the glasshouse and field experiments, plants were harvested during late pod-fill prior to the onset of senescence.

Physical recovery of roots (glasshouse and field)

Shoots were excised at ground level and either the substrate was removed from pots, or all the soil in replicated 0.32 m^2 microplot areas was dug to a depth of 25 cm. As many fragments of nodulated roots as possible were then removed from the rooting medium. The shoot and recovered roots and nodules were dried at 70 °C, weighed and analyzed for total N content.

Dilution of ^{15}N-enriched soil (glasshouse)

Shoot residues (15 g) of lupine (*Lupinus albus*) enriched in ^{15}N (6 atom %) were mixed thoroughly in the soil in each pot. The pots were kept moist and were left in the glasshouse (25 °C day/18 °C night) for 6 weeks prior to commencement of experimentation, in an attempt to ensure that the ^{15}N was fully incorporated in the soil organic fraction. Belowground contributions of fixed N were calculated on the basis of the observed 'dilution' of soil ^{15}N relative to a wheat (*Triticum aestivum*, cv. Janz) or unplanted control (after Poth *et al.*, 1986). Leachates were collected in drip trays and returned to the pots to minimize losses of ^{15}N. At plant harvest, shoots were excised, the soil was removed from each pot and roots were recovered. The total soil weight was determined and sub samples collected for later analysis.

N mass balance (glasshouse)

Estimates of legume BGN based on N mass balance were calculated from dry weight determinations and N analyses of collected shoot, root, and soil material from the ^{15}N-enriched soil experiment described above.

In situ ^{15}N shoot-labelling (glasshouse and field)

Urea enriched in ^{15}N (98 atm %) was supplied to the shoot either via a cut petiole (chickpea) or a leaf flap (fababean) on either three (glasshouse) or five occasions (field) prior to flowering. Each leaf-flap was cut as a narrow "V" underwater with the end of the "V" centered on the mid vein, close to the leaf tip. The leaf-flap or cut petiole was placed in a small tube containing 0.2 mL urea solution and kept in place with a small amount of teristat (blue-tac) putty. This also served to seal the top of the tube to prevent

evaporative losses and to attach the tube to a small wooden stake placed next to the plant. Glasshouse plants were fed 8.3 mg ^{15}N/pot (3 x 0.2 mL of 0.5% urea solution per plant). In the field experiment, all plants within the microplots were fed 1 mL (5 x 0.2 mL) of the enriched urea solution (16.1 and 18.4 mg ^{15}N applied to chickpea and fababean, respectively). The fed petioles and leaves were removed 2 weeks after feeding. Any abscised shoot material was removed within 48 h and retained for analysis. At harvest the shoots were excised, soil was totally removed from pots or field microplots (to 25-cm depth, then cored 25–45 cm), roots were recovered, total soil weight determined, and sub samples collected for analysis. It was assumed that all ^{15}N excess detected in the soil originated only from ^{15}N-enriched root material.

Estimates of BGN were subsequently calculated from the resulting N and ^{15}N data using three different approaches. The first approach assumed that the specific enrichment of recovered root material (i.e. mg ^{15}N/g root N) was representative of the unrecovered root-derived N still remaining in the soil (Rochester *et al.*, 1998; Russell and Fillery 1996a). In the second approach the ^{15}N data were adjusted to account for differences in the enrichments of unnodulated root and nodulated root (experimentally determined enrichment ratios were 1:12 for fababean and 1:56 for chickpea) and BGN estimates were recalculated. In the third approach, the amounts of ^{15}N label partitioned above- and below-ground (recovered roots + soil) were calculated, and BGN was estimated on the basis of the assumption of uniform translocation and partitioning of both labelled and unlabelled N to all plant parts.

Analyses and calculations

Plant and soil materials from the glasshouse and field trials were dried, weighed and roughly ground in a Wiley mill, sub sampled, and then finely ground with a ring grinder. The total N and ^{15}N contents of the dried ground samples were determined by combustion using an automatic N and C analyzer interfaced with a 20-20 stable isotope mass spectrometer (Europa Scientific). The ^{15}N data were expressed as Ò-^{15}N or parts per thousand (‰) relative to ^{15}N composition of atmospheric N_2 (i.e. 0.3663 atom % ^{15}N) using the following equation:

$$\text{Ò}^{15}\text{N} = 1000 \times \frac{(\text{atom \% } ^{15}\text{N}_{\text{sample}} - 0.3663)}{0.3663}$$

The content of excess ^{15}N (enrichment above natural abundance) was calculated by comparing the ^{15}N composition of enriched plant and soil samples with matching natural abundance material (unenriched controls).

Results and Discussion

Glasshouse studies

Physical recovery of roots

Estimates of BGN based on the physical recovery of roots (Figure 1) from the potting mix were made on three occasions. The amount of N recovered in roots and nodules represented between 9 and 12% of total plant N (mean 10%) for chickpea and between 10 and 19% (mean 13%) for fababean. The relative importance of nodule N appeared to differ between the two species, with nodules contributing a much higher proportion of BGN of chickpea (65%) compared to fababean (26%).

Dilution of ^{15}N-enriched soil

Belowground N of the two legumes was estimated by the ^{15}N soil-dilution method (Table 1) proposed by Poth *et al.* (1986). The ^{15}N enrichments of soil in the legume pots were related to the enrichment of the wheat soil to determine % soil N derived from N_2 fixation. The P fix values determined for the legumes were then used to adjust the values of soil N derived from N_2 fixation to root-derived N in soil. Estimates of BGN calculated in this way ranged from 11% (fababean) to 52% (chickpea). Comparison of the ^{15}N enrichments of the legume shoots (chickpea 545‰, fababean 210‰), with the wheat control (3,976‰) indicated significant contributions of fixed N for growth (the proportion of legume N derived from N_2 fixation, %Ndfa (Rochester *et al.*, 1998), was calculated to be 86 and 95% for chickpea and fababean, respectively). It was, therefore, surprising to find that the enrichment of the fababean soil (452‰) was similar to that of wheat soil, the non-N_2-fixing control (454‰). It was, however, lower than that measured in soil in the unplanted pot (513‰). In contrast to this observation, the low level of enrichment detected in the chickpea soil (401‰) implied dilution of ^{15}N by root-derived fixed N.

N mass balance

Data from the enriched-soil study were used to construct N budgets for both legume species (Table 2). Soil N present in each pot at the beginning of the experiment was subtracted from soil N measured at final harvest to determine any net change during the course of plant growth. This was added to N measured in the recovered roots to determine BGN (Table 2, 3). Values were calculated to be 30 and 52% for fababean and chickpea, respectively (Table 2).

In situ ^{15}N shoot-labelling

Recovery of fed ^{15}N in the shoot, roots and soil ranged from 76% (chickpea) to 90% (fababean). The ^{15}N abundance of the potting mix was significantly enriched following shoot-labelling of fababean (42‰) and chickpea (53‰) compared to soil in the untreated controls (3‰). The ^{15}N enrichments of the crown roots (including crown root, tap

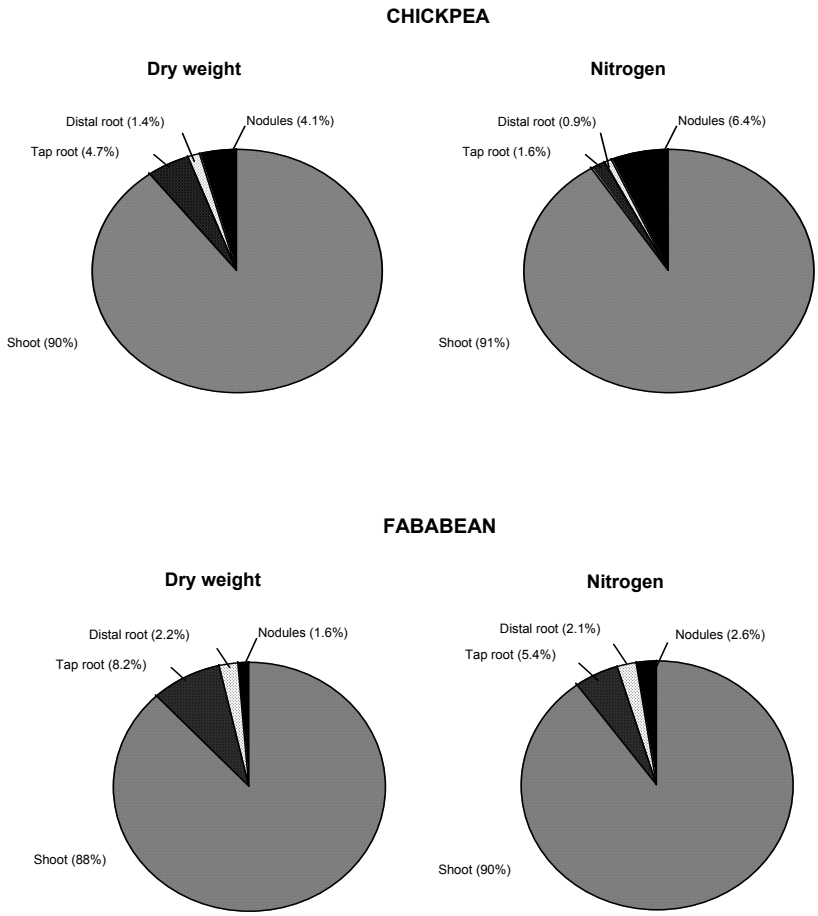

Figure 1. Dry weight and N Partitioning in Faba bean and Chickpea plant grown in glasshouse.

root and major laterals), and distal roots (minor laterals and fine roots) were identical (355‰) for fababean, but differed in the case of chickpea (crown roots, 317‰; distal roots, 241‰).

Assuming that the ^{15}N and N characteristics of the recovered root material was representative of the unrecovered root-derived N remaining in the soil (Rochester *et al.*, 1998; Russell and Fillery, 1996b), BGN was calculated to represent 39 and 53% of total plant N for fababean and chickpea, respectively (Table 3). Estimates of BGN were similar for fababean (37%), and slightly lower for chickpea (42%) if it was assumed that the unrecovered roots were unnodulated and the data were adjusted to account for the likely difference in enrichment between nodulated and unnodulated roots. Values were again similar (33% for fababean and 43% for chickpea) if it was assumed that the percentage distribution of ^{15}N label measured in the shoots, recovered roots and soil reflected the above- and below-ground partitioning of plant N.

Field study

Physical recovery of roots

It was exceedingly difficult to recover intact root fragments from the heavy textured soil (58% clay, 22% silt, 20% sand) at the field site. The amounts of N present in the roots removed from the soil (0.13 and 0.16 g N per microplot for fababean and chickpea, respectively) were dwarfed by the amounts of N measured in the shoots (5.52 and 2.18 g N for fababean and chickpea, respectively). Subsequent estimates of BGN represented only 2% (fababean) to 7% (chickpea) of the total plant N recovered.

In situ ^{15}N shoot-labelling

Recovery of ^{15}N in harvested plant parts and soil accounted for 91 to 92% of the ^{15}N enriched urea-N applied.

Table 1. Below-ground N (BGN) as a percentage of total plant-derived N for fababean and chickpea grown in ^{15}N-enriched soil in 23 L pots in a glasshouse, using ^{15}N dilution [Soil enrichment was achieved by adding 15 g lupin shoot residues (6% ^{15}N a.e.) to each 23 L pot of soil; values (± s.e.) expressed on per pot basis]

Species	Root N equivalent in soil (g)	Total BGN (g)	Total plant N (g)	BGN (% of total)
Fababean	0.05 ± 0.17	0.40 ± 0.19	3.38 ± 0.31	11
Chickpea	1.64 ± 0.44	1.84 ± 0.44	3.46 ± 0.33	52

Table 2. Below-ground N (BGN) as a percentage of total plant-derived N for fababean and chickpea grown in ^{15}N-enriched soil in 23 L pots in a glasshouse, using an N-balance approach [Soil enrichment was achieved by adding 15 g lupin shoot residues (6% ^{15}N a.e.) to each 23 L pot of soil; values (± s.e.) expressed on per pot basis]

Species	Shoot N (g)	Recovered root N (g)	Gain/loss soil N (g)	Total plant N (g)	BGN (% of total)
Fababean	3.0 ± 0.14	0.35 ± 0.03	0.96 ± 0.23	4.29 ± 0.35	30
Chickpea	1.6 ± 0.12	0.20 ± 0.01	1.64 ± 0.38	3.46 ± 0.26	52

Table 3. Below-ground N (BGN) as a percentage of total plant-derived N for ^{15}N-shoot labelled fababean and chickpea grown in 23 L pots in a glasshouse [All 6 plants/pot were fed with a total of 0.6 mL ^{15}N-labelled urea (0.5% solution, 98% enrichment); values (± s.e.) expressed on per pot basis]

Species	Shoot N (g)	Recovered root N (g)	Root N equivalent in soil (g)	Total plant N (g)	BGN (% of total)
Fababean	2.81 ± 0.13	0.68 ± 0.05	1.05 ± 0.04	4.47 ± 0.31	39
Chickpea	2.00 ± 0.15	0.26 ± 0.02	1.86 ± 0.17	4.23 ± 0.01	53

Table 4. Below-ground N (BGN) as a percentage of total plant-derived N for ^{15}N-shoot labelled fababean and chickpea grown in (0.32m^2) microplots in a field at peak biomass [All plants (fababean 8 and chickpea 7/microplot) were fed five times of 0.2mL of ^{15}N-labelled urea (0.5% solution, 98% enrichment); values (± s.e.) are per microplot]

Species	Shoot + fallen leaves N (g)	Recovered root N (g)	Root N equivalent in soil (g)	Total plant N (g)	BGN (% of total)
Fababean	5.52 ± 0.70	0.13 ± 0.01	1.70 ± 0.25	7.35 ± 0.79	25
Chickpea	2.18 ± 0.20	0.16 ± 0.02	7.16 ± 1.01	9.50 ± 1.21	77

Enrichments in the 0- to 25- and 25- to 45-cm layers of soil removed from the microplots were 18‰ and 8.7‰ under fababean, and 30‰ and 8.8‰ under chickpea, respectively, compared with 6.1 to 6.3‰ in soil collected from outside the microplots. The enrichments of fababean material sampled from the microplots were 568‰ (shoot) and 674‰ (root), and the values for chickpea were 705‰ (shoot) and 331‰ (root) compared to 0 to 3.5‰ in plants sampled from the surrounding unenriched crop. Calculations using the relative ^{15}N excess of the recovered roots and the ^{15}N enrichment of soil indicated that BGN represented 25% of total crop N for fababean and 77% for chickpea (Table 4).

The high BGN value for chickpea was caused by a combination of a low ^{15}N enrichment of recovered roots and a relatively high enrichment of the 0 to 25 cm soil N. Neither the 'adjusted' nor '^{15}N balance' approaches had a large effect on estimates of BGN for fababean (24 and 29%), but both modifications of the shoot-labelling technique reduced the values calculated for chickpea (68 and 60%). It appears that the most error-prone and inaccurate method for estimating BGN is the physical recovery of roots. The values obtained with physical recovery (10–13% of whole plant N in the glasshouse, and 2–7% in the field) were only a fraction of those obtained using the other methodologies. This should be expected since even if it were possible to completely recover intact root systems, such measures would not include N derived from the turnover of nodules and roots or root exudations that occur during growth.

With the exception of the soil ^{15}N-dilution method for fababean, most techniques used in the glasshouse studies gave reasonably similar determinations of BGN. Averaged across all estimates (other than physical recovery), BGN of glasshouse-grown plants represented 30% of total plant N for fababean and 48% for chickpea. Although the ^{15}N-based methods used in the field study have questionable assumptions with built-in errors, it was reassuring that all three calculations provided estimates that were similar to each other (24–29% and 26% mean for fababean, 60–77% and 68% mean for chickpea), and were comparable to those obtained under very different conditions in the glasshouse (Table 1). However, it is unlikely that there is a single value for BGN for a species, and it is reasonable to assume that the root:shoot ratio is influenced by growth conditions or stress and for species to respond in differing ways. This presumably explains why estimates of BGN for chickpea in the field were slightly higher than detected in the glasshouse.

Conclusions

Field and glasshouse studies of fababean and chickpea indicated that much higher proportions of total legume N are associated with, or derived from, roots than previously believed. It is clear that BGN represents an important pool of residual N that has been grossly underestimated or ignored in past calculations of rotational N budgets.

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