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Nitrogen isotope fractionation in the fodder tree legume tagasaste (*Chamaecytisus proliferus*) and assessment of N₂ fixation inputs in deep sandy soils of Western Australia

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Abstract. Nitrogen (N) isotope fractionation and symbiotic N fixation were investigated in the shrub legume tagasaste, growing in the glasshouse and field. In a pot study of effectively nodulated plants supplied with 0, 1, 5 and 10 mM nitrate [stable isotope ¹⁵N ($\delta^{15}\text{N}$) of 3.45‰], the $\delta^{15}\text{N}$ of dry matter N of fully symbiotic cultures indicated a greater isotope fractionation during distribution of N between nodules, stems, leaves and roots than for N₂ fixation itself, with whole-plant $\delta^{15}\text{N}$ being near zero (–0.46 to 0.42‰). Regardless of whether plants were field-grown, pot-cultured, fixing N₂ or utilising mineral N, woody stems were depleted in ¹⁵N relative to all other plant parts. The similar orders of ranking of $\delta^{15}\text{N}$ for plant components of the nitrate-treated and fully symbiotic plants, and a general increase in $\delta^{15}\text{N}$ as plants were exposed to increasing concentrations of nitrate, indicated that N isotope fractionation can be accounted for, and thus not undermine ¹⁵N natural abundance as means of measuring N₂ fixation inputs in tagasaste trees. In pot culture the percentage of plant N derived from the atmosphere (%Nd_fa) by symbiotic N₂ fixation fell from 85 to 37% when the nitrate supply was increased from 1 to 10 mM, with evidence of nitrate N being preferentially allocated to roots. $\delta^{15}\text{N}$ natural abundance assessments of N₂ fixation of 4-year-old trees of field-grown tagasaste in alley (550 trees ha^{–1}) or plantation (2330 trees ha^{–1}) spacing were undertaken at a study site at Moora, Western Australia, over a 2-year period of shoot regrowth (coppicing). Cumulative N yields and %Nd_fa were similar for trees of alley and plantation spacing, with much less coppice N accumulation in the first compared to the second year after cutting. Scaling values from a tree to plot area basis, and using a mean %Nd_fa value of 83% for all trees at the site, inputs of fixed N into current biomass plus fallen litter over the 2 years of coppicing were calculated to be 83 kg N ha^{–1} year^{–1} for the alley and 390 kg N ha^{–1} year^{–1} for the plantation spacing. Although the plantation tagasaste fixed 587 kg N ha^{–1} in the second year, close to the maximum value reported in the literature for any N₂-fixing system, this should not be seen as typical where the trees are used for animal production, since grazing and cutting management will substantially reduce productivity and N₂ fixation input.

Keywords: agroforestry, soil N cycling, nitrate, ¹⁵N natural abundance.

Introduction

Extensive research in recent years (see review of Hogberg 1997) has improved our understanding of natural variations in the abundance of the stable isotope ¹⁵N ($\delta^{15}\text{N}$) within plant–soil systems, and how these might be gainfully exploited in field studies of plant N acquisition. Since atmospheric N₂ has a constant ¹⁵N abundance (0.3663 atom%) it is used as the standard (0‰) against which natural variations in $\delta^{15}\text{N}$ are measured, and although soil $\delta^{15}\text{N}$ is much more variable than atmospheric N₂, there is a strong tendency for it to be lightly enriched in ¹⁵N (0.5–5‰) relative to the atmosphere. In annual agricultural ecosystems, these variations in $\delta^{15}\text{N}$ have been used to estimate the percentage of legume N

derived from the atmosphere (%Nd_fa) as opposed to soil N sources (Peoples *et al.* 1989). In the case of perennial legumes and in natural ecosystems, the methodology has many more complications (Handley and Scrimgeour 1997), although $\delta^{15}\text{N}$ -based investigations have proved useful for examining plant N acquisition strategies of shrubs and trees in some field studies (e.g. Hogberg 1990; Stewart *et al.* 1993; Pate *et al.* 1998). The complications principally relate to fractionation of N isotopes as N moves from one pool to another within the soil (Hogberg 1997) and within the plant (Yoneyama 1995), and so before one can confidently use $\delta^{15}\text{N}$ to assess N acquisition strategies of perennial plants in a given ecosystem, an examination of the variations of ¹⁵N within these pools is required.

Abbreviations used: DAS, days after sowing; DM, dry matter; %Nd_fa, percentage of plant N derived from the atmosphere; $\delta^{15}\text{N}$, stable isotope ¹⁵N.

In this paper we investigate the use of the ^{15}N natural abundance technique for assessing symbiotic N_2 fixation of the perennial fodder shrub legume, tagasaste (*Chamaecytisus proliferus* Link), which is grown on deep sands on some 50 000 ha of farmland in Western Australia. Here it plays an important role as a supplementary source of stock feed during autumn, and, grown as wide-spaced alleys or dense plantations, might also ameliorate hydrological imbalance and provide inputs of biologically fixed N (Lefroy and Stirzaker 1999).

Our studies include a pot trial to investigate N isotope fractionation and N_2 fixation in nodulated young trees, and field studies to monitor N_2 fixation of coppicing alley- and plantation-spacing tagasaste growing on deep sand at Moora, Western Australia. The study complements recent investigations of plant production and concomitant water utilisation by the species at the same site (Lefroy and Stirzaker 1999; Lefroy *et al.* 2001) as part of a series of comparisons of hydrological and N balances of pasture, crop, tagasaste and natural vegetation systems in the region (Pate and Bell 1999; Pate and Dawson 1999).

Materials and methods

Glasshouse studies

^{15}N isotope fractionation of tagasaste was examined in pots in a naturally lit and heated glasshouse at the University of Western Australia. Effectively nodulated young trees were grown in the presence or absence of a defined source of inorganic N, and shoots, roots and leaves analysed for $\delta^{15}\text{N}$. We thus hoped to determine how best to apply the ^{15}N natural abundance technique to estimate symbiotic N_2 fixation of this species under field conditions.

Plastic pots of 10 L capacity containing heat-sterilised nutrient-free silica sand were each sown with five tagasaste seeds in autumn (April 1997) and thinned to three seedlings per pot. Seeds were inoculated at sowing with commercial inoculant, *Bradyrhizobium* strain CC1502 (Bio-Care Technology Pty Ltd, Somersby, NSW). Seedlings were watered twice weekly with a nutrient solution that was either N-free or contained 1, 5 or 10 mM KNO_3^- . The volume of nutrient solution added on each occasion was sufficient to flush out previously applied nutrients and thus prevent build-up of salts in the rooting medium. Each NO_3^- treatment involved three replicate pots, while six replicates for the N-free treatment permitted a second harvest of tagasaste 4 months after conclusion of the NO_3^- feeding trial.

The $\delta^{15}\text{N}$ of the supplied NO_3^- (3.45‰) was similar to that shown in a preliminary study of soil mineral N components (3.1–4.5‰) at the field study site. Young trees from all NO_3^- -treated plants and half of the N-free pots were harvested in late August 1997, 145 d after sowing (DAS). The remaining N-free cultures were harvested in December (269 DAS). Plants were separated into stems, leaves, roots and nodules, and each of these components bulked on a per pot basis. Fallen leaves were collected at the later harvest of the N-free pots. All plant samples were oven-dried at 70°C, weighed and ground in a ball mill prior to analysis for total N and $\delta^{15}\text{N}$.

Field studies at Moora

Details of the site on the Brown property off Agaton Rd near Moora (116°40' S, 30°45' E) are provided by Lefroy and Stirzaker (1999). The soil is a highly uniform siliceous sand (Dystric Xerosamment; McArthur 1991), overlaying clay at 10 m depth and a perched non-

saline water table at 5 m. The site was established in 1992 as five replicate pairs of plots within an 8-ha block of land, in either a plantation spacing with rows of trees 6 m apart (final tree density of 2330 trees ha^{-1}) or in alley spacing with rows 30 m apart (550 trees ha^{-1}). Rhizobia capable of producing effective nodules on tagasaste are widespread in soils of southern Australia (Gault *et al.* 1994). Areas between rows of the alley spacings were sown annually with either a cereal or lupin crop (alley-crop spacing), or as a regularly sprayed chemical fallow that prevented revegetation with annual species (alley-fallow spacing). Each spacing (plantation, alley-crop and alley-fallow) involved two replicate 50 × 160 m plots.

In late autumn (May) of 1996, the 4-year-old tagasaste trees were mechanically pruned to 0.6 m height. Sheep were then introduced to graze the prunings until the establishment of the alley-crop and alley-fallow spacings the following June. Thereafter, all trees grew without further pruning or grazing. The alley-crop spacings were sown with narrow leaved lupins (*L. angustifolius* cv. Gungurru in 1996 and cv. Merrit in 1998) alternating with oats (*Avena sativa* cv. Toodyay) in 1997. Rainfall for the first 2 calendar years was 445 mm (1996) and 431 mm (1997), while 88 mm of rain was recorded between January and May of 1998.

Above-ground tagasaste biomass was harvested on a monthly basis in the first year, but changed to 3-monthly thereafter so as not to reduce tree densities to the extent that plant water relations would be affected. To assess rates of growth and N accumulation, all coppice regrowth on four randomly selected trees from each plot was cut back to the initial prune height of 0.6 m at each harvest. The coppice fresh weight was recorded immediately, and representative subsamples weighed and taken back to the laboratory for drying and determination of fresh weight:dry weight ratio in order to assess coppice dry matter (DM) yields. For the purposes of this study, data for alley-crop and alley-fallow plots were pooled and are referred to hereafter as 'alley' spacing. Dry biomass was fed through a chipper before being further subsampled and analysed for total and $\delta^{15}\text{N}$.

In addition to sampling whole coppice, a single actively-growing 3–10-cm apical region of a coppice shoot was selected from random positions in the canopy of each of 30 plants from each of the six rows in an alley plot, or from each of six randomly selected rows of the 30-row plantation plots. These monthly harvests were used to assess current N_2 fixation in newly acquired and mobilised N allocated to shoot growth over specific periods, in contrast to the cumulative N yields encompassed in the whole-coppice samples.

At the end of the study (May 1998), total accumulation of N by alley and plantation trees during the 2-year period of coppice regeneration was assessed using a mean shoot:root biomass dry weight ratio of 2.7 (recorded by J. S. Pate and E. C. Lefroy, unpublished data) for excavated trees, and applied to both the initial and final harvest times of the present study. Increments in total DM tree^{-1} were also estimated, from the DM ratio of coppice regrowth to parent lower trunk biomass at final harvest. Using values for %N in DM of coppice shoot, stump and roots, N increments of these parts on a per tree basis could be calculated.

Tagasaste leaf litter was also collected at final harvest. In the alley spacings a series of 1-m² quadrats was used to sample beneath tree canopies and also to the outer limits (2.3–2.8 m) at which litter had accumulated. Comparable collections in plantation tree rows were taken from the middle of a tree row to the mid-regions between the adjacent rows of trees. Based on tree planting density, accumulation of litter biomass was estimated on both a metre row and per tree basis.

Measurement of symbiotic N_2 fixation using the ^{15}N natural abundance technique

Measurement of N_2 fixation closely followed the methods described by Unkovich *et al.* (1994, 1997). Plant dependence on %Nd_{fa} was estimated by comparing the $\delta^{15}\text{N}$ of plant material sampled from the N_2 -fixing legume with that of either the fed source mineral N (pot NO_3^-

study), or selected non-leguminous reference plants (field studies). In both cases, allowance was made for any isotope fractionation occurring as fixed N subsequently exchanged between plant parts. The extent of such fractionation was assessed using $\delta^{15}\text{N}$ obtained from nodulated pot-cultured plants raised in the absence of combined N. These $\delta^{15}\text{N}$ values are referred to hereafter as 'B' values for the various plant parts.

Thus for pot-grown plants;

$$\% \text{Ndfa} = \frac{(\delta^{15}\text{N of } \text{NO}_3^- \text{ supplied}) - (\delta^{15}\text{N of selected part of } \text{NO}_3^- \text{-fed tagasaste})}{(\delta^{15}\text{N of } \text{NO}_3^- \text{ supplied}) - (\text{'B' value of matching plant part of fully symbiotic tagasaste})} \times \frac{100}{1}$$

and for field-grown plants;

$$\% \text{Ndfa} = \frac{(\delta^{15}\text{N of shoots of reference non-legumes}) - (\delta^{15}\text{N of shoots of tagasaste})}{\delta^{15}\text{N of shoots of reference non-legumes} - (\text{'B' value of tagasaste shoot})} \times \frac{100}{1}$$

In the field studies of tagasaste N_2 fixation, $\delta^{15}\text{N}$ for shoots of the oldest (269 DAS) pot-grown fully symbiotic tagasaste (1.5 m tall) were used as the B value, while reference plants used to sample $\delta^{15}\text{N}$ of plant-available soil N included the annual weed species, capeweed (*Arctotheca calendula* L.), wild radish (*Raphanus raphanistrum* L.) and mustard (*Brassica tournefortii* Gouan). From each plot, 5–10 whole shoots of each of these species were sampled for $\delta^{15}\text{N}$ analysis. Since these species were only available during the winter and spring seasons (June–November), shoot material was also sampled from two deep-rooted perennial non-legume reference species. One of these, the native herbaceous perennial *Psilostachyus*, was sparsely distributed across the plots but, since it was summer-active in its growth, it provided vital reference non-legume material for the period November–April, when annual weeds were not available. The number of plants of this species varied between plots from 1–2 to 15–20, but for each plot, shoot tips considered current or recent sinks for N were taken from each individual plant and bulked for each plot. Recently formed leaves of two individuals of the other species, *Eucalyptus totidiana*, a phraeatophytic tree growing adjacent to the study plots, were also sampled.

An earlier study (Pate *et al.* 1993) had shown *P. polystachyus* to be nitrophilous and therefore likely to be subsisting mostly on nitrate in soil and groundwater. Samples of nitrate-rich ground water ($6.6 \pm 0.6 \text{ mg L}^{-1} \text{ NO}_3^- \text{-N}$) were collected from bores in the plots and subjected to ^{15}N assay using distillation of samples with Devarda's alloy and magnesium oxide, and collection of the resultant NH_4^+ on zeolite (Velinsky *et al.* 1989) prior to isotope ratio analysis by continuous flow mass spectrometry.

$\delta^{15}\text{N}$ and %N analysis of all samples were conducted using an automated CN analyser connected to a high precision isotope ratio mass spectrometer (VG Isogas SIRA 9 or Europa Scientific 20/20, Crewe, UK). Analytical procedures and estimates of likely errors of measurement were as detailed in Unkovich *et al.* (1994).

Results

Glasshouse studies of N isotope fractionation in symbiotically-dependent and nitrate-fed young trees

$\delta^{15}\text{N}$ for shoots, leaves, stems, roots, nodules and whole plants, grown with or without nitrate and harvested at 145 DAS, are shown in Fig. 1, and for the later harvest (269 DAS) of symbiotically-dependent plants, in Table 1. Plants dependent solely on fixed N showed a greater isotope

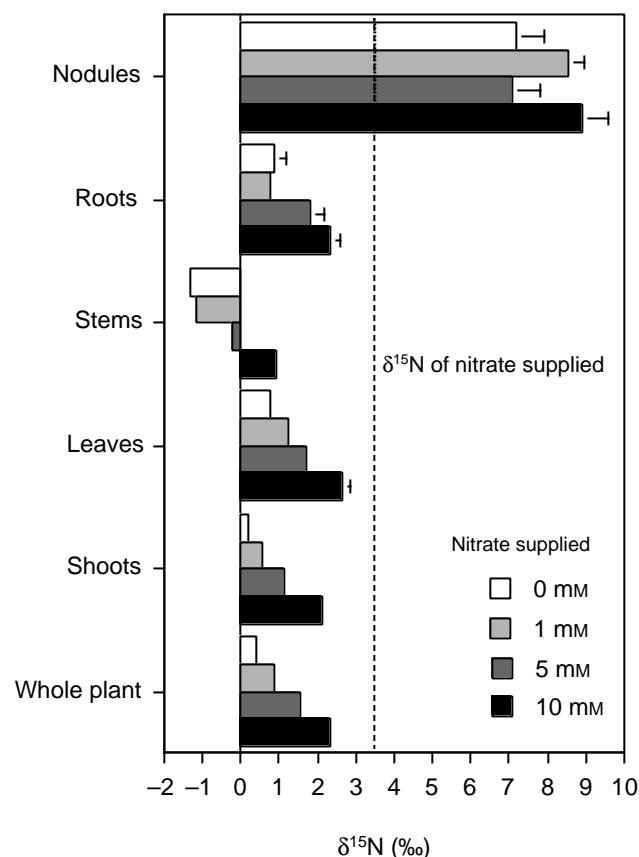


Fig. 1. $\delta^{15}\text{N}$ of component parts and calculated values for whole plants of young trees of N_2 -fixing tagasaste in sand culture in a glasshouse at the University of Western Australia, grown with 0 (fully symbiotic), 1, 5 or 10 mM nitrate and harvested 145 DAS. Error bars are s.e. of mean. Derived %Ndfa values are shown in Table 2.

Table 1. $\delta^{15}\text{N}$ of dry matter total N of symbiotically N-dependent tagasaste grown in N-free glasshouse sand culture and harvested 269 DAS

These are used as 'B' values when assessing %Ndfa of field-grown tagasaste. Comparable values for an earlier harvest (145 DAS) are shown in Fig. 1. Whole-plant values do not include fallen leaves

	$\delta^{15}\text{N}$ (‰)	s.e.
Whole plant	-0.47	0.04
Shoot	-0.48	0.06
Leaves	0.53	0.10
Stem	-1.67	0.20
Nodulated roots	-0.53	0.20
Fallen leaves	0.07	0.10

fractionation during N distribution between nodules, stems, leaves and roots than for N_2 fixation itself, with nodules accumulating ^{15}N (7.2‰ at 145 DAS) compared to stems (-1.5‰), leaves or roots (both ca 0.8‰). The same order of ranking of $\delta^{15}\text{N}$ for plant parts was also evident for nitrate-fed plants (Fig. 1), even though there was a general tendency

towards more positive $\delta^{15}\text{N}$ as nitrate supply was increased and trees incorporated greater amounts of the 3.45‰ nitrate N source.

The values in Fig. 1 were used to estimate %Ndfa (Table 2) for the component parts of the young trees harvested from each nitrate treatment. Resulting data for whole-plant N showed a substantial decrease in proportional dependence on N_2 fixation (85% down to 37%) as the nitrate supply increased. Similar trends were recorded for the %Ndfa of shoots and leaves. However, regardless of concentration of nitrate supplied, the isotope data indicated that nitrate N appeared to be sequestered especially by roots.

Growth and N accumulation in coppicing alley and plantation plots

In the field study, variations in amounts of coppice DM tree⁻¹ were large between and within plots of alley and plantation spacings at each time of sampling (Fig. 2A), so general courses of coppice growth are shown using curves of best fit. Combining this information with %N in DM of corresponding coppice biomass samples (Fig. 2B), cumulative N yields in coppice biomass of alley and plantation trees were derived (Fig. 2C). The data indicated relatively slow coppice growth and N accumulation in the first 8 months or so as trees recovered from cutting, followed by a rapid escalation of growth which resulted in an almost 4-fold increase in accumulated DM and N over the second season of growth. Apart from a tendency for DM and N to accumulate at a faster rate in plantation compared to alley trees during winter and spring of the second season (1997), the trees in the two planting spacings showed remarkably similar patterns of growth and N acquisition overall (Fig. 2C).

There was a general trend for coppice biomass to be richer in N (3–4.5% N in DM) in the first season after cutting (Fig. 2B), as to be expected from progressive accumulation of woody material as stems became secondarily thickened and shed N-rich foliage. There was also a tendency in both plantation and alleys for N concentration in coppice DM to be particularly low during late summer and autumn (1996/7 and 1997/8), coincident with stress-induced leaf fall.

Table 2. Estimates of %Ndfa in leaves, shoots, nodulated roots or whole plants of pot-cultured tagasaste fed 0, 1, 5 and 10 mM KNO₃ and harvested 145 DAS

Values in parentheses are standard error of the mean

Applied NO ₃ ⁻ (mm)	%Ndfa			
	Leaves	Shoots	Nodulated roots	Whole plant
0	100 (1.2)	100 (2.0)	100 (10.5)	100 (3.7)
1	83 (1.3)	89 (1.5)	64 (7.8)	85 (1.9)
5	64 (3.3)	71 (3.6)	36 (4.1)	63 (3.5)
10	30 (8.5)	40 (5.1)	25 (2.5)	37 (3.2)

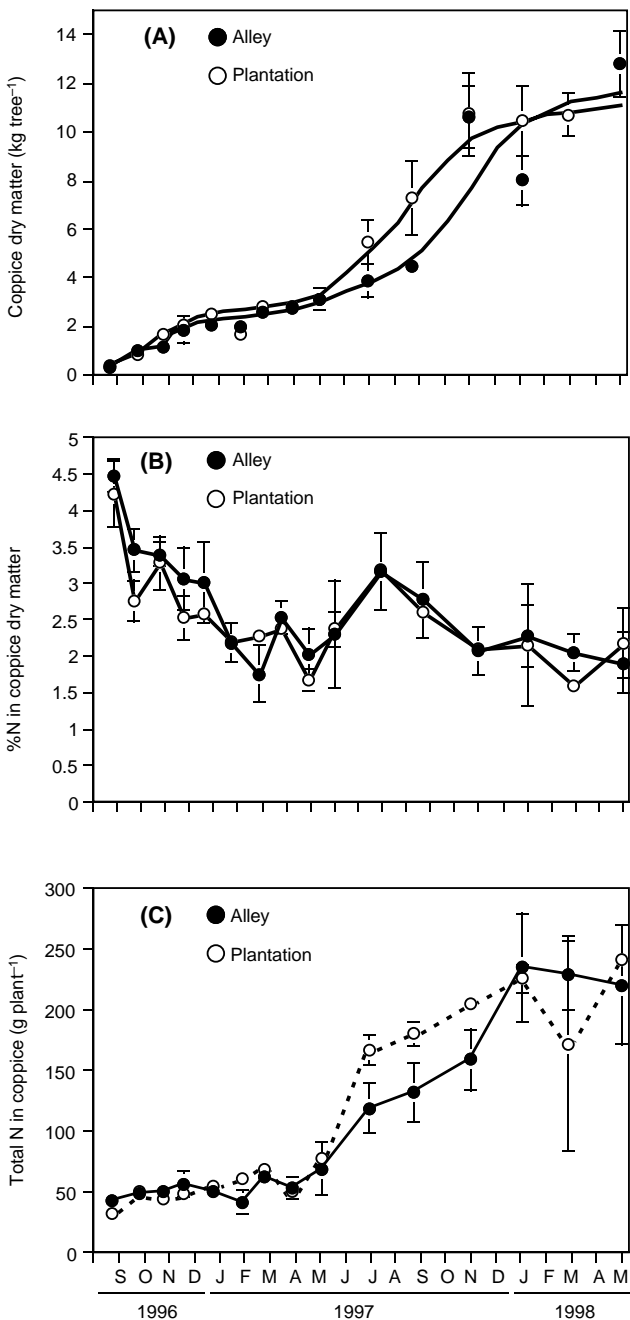


Fig. 2. Assessments of (A) cumulative dry matter, (B) %N in dry matter and (C) cumulative total N of coppice regrowth of trees of tagasaste, over a 2-year period after an initial cut (May 1996) when 4 years old. Note much faster growth and N gain in the second compared to the first season after cutting. Alley trees with annual crop or fallow between tree rows were grown at a density of 550 trees ha⁻¹, those in plantation spacing at 2330 trees ha⁻¹.

Conversely, %N increased sharply from the onset of rains in May through winter, to the following August/September when coppices were at peak leafiness and presumably capable of assimilating more than sufficient N to maintain current growth.

$\delta^{15}\text{N}$ signals of agroecosystem components and derivation of %Nd_{fa} for whole coppice and new shoot tip growth of tagasaste

Figure 3 summarises data for $\delta^{15}\text{N}$ of coppice of alley and plantation tagasaste, shoot total N of reference plants and corresponding %Nd_{fa} for trees of all treatments and planting spacings for the 2 years of study. Data sets for whole coppice DM (Fig. 3A) and coppice tip growth (Fig. 3B) showed relatively small changes in $\delta^{15}\text{N}$ of coppice N between harvest times, but a narrower and less positive range of values for whole coppice shoot N (−0.4 to 0.9‰) than for corresponding shoot tips (0.7 to 2.1‰).

Because of the sporadic distribution of reference species within and between plots, $\delta^{15}\text{N}$ values for reference plant

collections made across the study site were pooled for each time of sampling regardless of species concerned and location of collection. This common set of reference plant values was used for deriving the %Nd_{fa} values given for whole coppice and coppice shoot tips in Fig. 3. When deriving %Nd_{fa} for whole coppice N, the B value of −0.48‰ (Table 1) obtained for 269-d-old shoots of the symbiotically-dependent pot-grown plants was used, whereas when making similar estimates for shoot tip biomass, the B value of 0.53‰ (Table 2) obtained for leaf material of the same pot-cultured plants was employed.

The range in $\delta^{15}\text{N}$ for reference species (2.2–5.1‰) (Fig. 3) tended to be broader than for tagasaste but, averaging 3.72‰ (s.d. 0.78‰) across all species and harvest times, was high enough to obtain meaningful values for %Nd_{fa} of simultaneously sampled tagasaste. $\delta^{15}\text{N}$ for mature deep-rooted individuals of the perennial *Ptilotus* (range 2.9–5.1‰) were not appreciably different from those of shallower-rooted annual weeds (range 2.4–4.7‰). Monthly analysis of $\delta^{15}\text{N}$ of groundwater nitrate under the plots ranged from 1.5–4.8‰ over a 12-month period (February 1997–March 1998), averaging 2.52‰ (s.d. 0.85). Xylem sap N from the lower tap root of tagasaste (2.8–3.3‰), and summer shoot growth of *Eucalyptus todtiana* (3.7–4.0‰) also lay within the same range as that of *Ptilotus* and annual shallow-rooted weeds. These data collectively show that the nitrate-related $\delta^{15}\text{N}$ signals recorded for ground water and tagasaste sinker root xylem sap were not appreciably different from those displayed by the various non-N₂-fixing reference plants, providing some justification for using the pooled reference plant $\delta^{15}\text{N}$ data for annual weeds and *Ptilotus*. Because of the close similarity in $\delta^{15}\text{N}$ of alley and plantation tagasaste, means for these for each time of sampling were used to derive the %Nd_{fa} values shown in Fig. 3. The values thus denote overall performance of all trees at the site in terms of dependence of whole coppice or shoot tip growth on fixed N.

The $\delta^{15}\text{N}$ data indicated uniformly high (78–89%) dependence of whole coppice growth on fixed N₂ as opposed to soil N (Fig. 3A). The partner set of values for new shoot tip growth (Fig. 3B) were more variable (52–86%), with evidence of lower overall dependence on fixed N (66%) than was exhibited by whole coppice biomass (82%).

Time courses of accumulation of N from fixation and soil into tree biomass

The somewhat volatile total N accumulation curve shown in Fig. 4 reflects the difficulties encountered in sampling and subsampling of large shrubs or trees. Total amounts of N at final harvests of alley and plantation trees were similar (approximately 230 g N tree^{−1}), with the bulk of this derived from N₂ fixation. However, there was evidence of coppice in plantation plots accumulating N somewhat in advance of that of alley plots during winter/spring of 1997. This may have

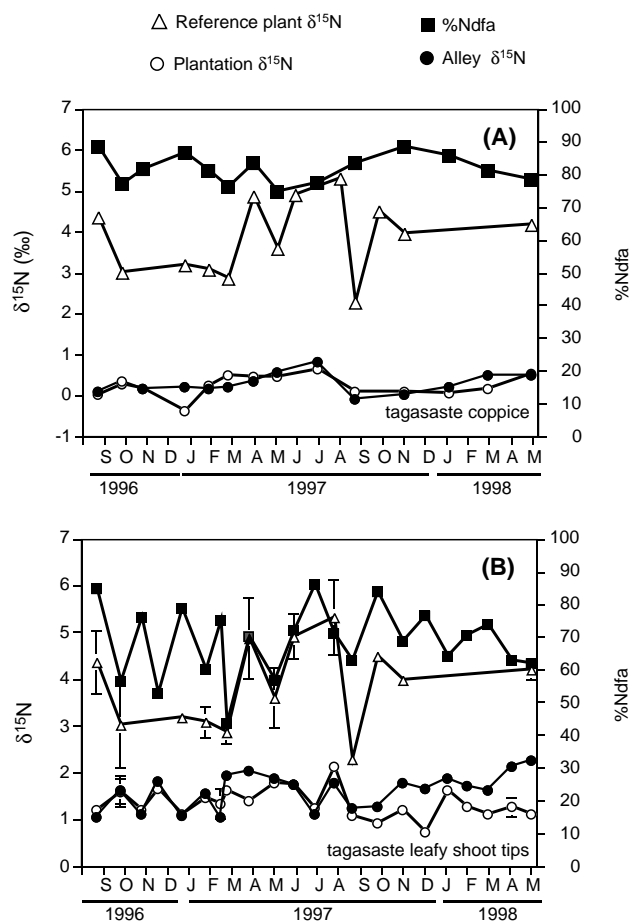


Fig. 3. $\delta^{15}\text{N}$ of total N of (A) whole coppice dry matter and (B) new leafy shoot growth sampled sequentially from coppice regrowth of trees of tagasaste after a first cut (May 1996) when 4 years old. Data are for alley (550 trees ha^{−1}) and plantation (2330 trees ha^{−1}) trees on a property near Moora, Western Australia. Mean $\delta^{15}\text{N}$ for non-legume reference plant (annual weeds and the deep rooted perennial *Ptilotus polystachyus*) shoot biomass collected across the study site are shown in A and B, and these were used to calculate %Nd_{fa} for plantation and alley trees in A and B.

been a result of the root activity of the alley-culture trees being disrupted in early winter by cultivation of the soil during seeding of the interspersed annual crop. Based on the cumulative N at final harvest shown for total coppice N in Fig. 4, mean %Nd_{fa} for plantation and alley plots was rated as 83%.

Rates of N accretion by alley and plantation trees are shown in Table 3. These values were derived from (a) amounts of total N in regrowth biomass at the end of the 2 years of coppicing (item 1, Table 3, derived from Fig. 4), and (b) estimates of increments in N contents of parent lower trunk material below the coppice shoots (item 2) and N of root biomass (item 3), as derived from ratios of dry matter and total N in shoot to root, and the DM ratio of coppice

regrowth to parent lower trunks (J. S. Pate, unpublished data). Totals for the above three items amounted to 306 and 339 g N tree⁻¹ for alley and plantation, respectively. With 550 trees ha⁻¹ in the alley configuration, and 2330 ha⁻¹ in plantation spacing, these values would translate to 168 kg N ha⁻¹ in total tree biomass of the alley spacings and 790 kg N ha⁻¹ in the plantations over 2 years.

The 3-cm-deep carpet of litter accumulating under the tagasaste was composed principally of fallen foliage, but also contained seeds and fine twig material. Concentrations of N in this litter were relatively high (1.5–2.0% N in DM) and, with litter densities in the range of 18–25 kg DM tree⁻¹, amounts of litter N accumulated under each tree turned out to be surprisingly large (item 4, Table 3), at 179 g N alley tree⁻¹ (98 kg N ha⁻¹) and 192 g N plantation tree⁻¹ (447 kg N ha⁻¹). Assuming that this litter had accumulated evenly across the 6 years of tree growth and that mineralisation of litter N had been negligible, one third of the above amounts (33 and 149 kg N ha⁻¹, respectively) are calculated to have been returned to the ground surface as litter during the 2-year study period.

A proportion of these litter inputs can be added to the annual rates of fixation ha⁻¹ using the mean value of 83%Nd_{fa} for both plantation and alley, and the data for coppice N shown in Fig. 4. The total N increment of 201 kg N ha⁻¹ as living biomass plus litter fall associated with 2 years of coppicing of alleys equates to a mean fixation input of 83 kg N ha⁻¹ year⁻¹, whereas trees at plantation spacing would be expected to have fixed an average of 390 kg N fixed ha⁻¹ year⁻¹.

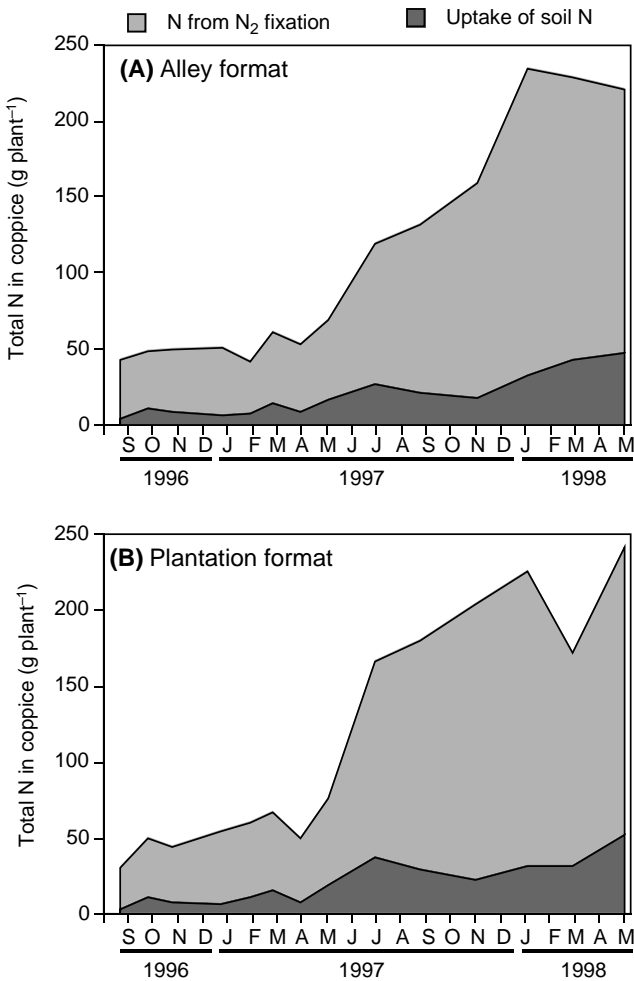


Fig. 4. Cumulative total N in coppice regrowth of alley- and plantation-grown trees of tagasaste for a 2-year period of recovery from a first cut (May 1996) when trees were 4 years old. Data derived from Figs 2 and 3, with values for %Nd_{fa} of alley and plantation plots used to distinguish proportions of N derived from fixation and uptake of combined N of soil or ground water.

Table 3. Net N balances of alley- and plantation-spaced tagasaste trees over a 2-year period of coppice regrowth following cutting of virgin trees when 4 years old (Moora, Western Australia; June 1996–June 1998)

Coppice shoot regrowth values are derived from Fig. 2c. Lower stem and root biomass increment figures are based on shoot to root dry weight ratios of uncut 4-year-old trees and coppicing trees 18 months after cutting, dry weight ratios of coppice regrowth to basal biomass of parent lower trunks, and %N in dry matter of these components (J. S. Pate and E. C. Lefroy, unpublished data). Accumulated litter assessed by sampling across the litter fall area of alley and plantation trees, measuring litter dry weights and %N, and estimating cumulative litter fall in terms of g N tree⁻¹

Quantity	Alley spacing	Plantation spacing
	(g total N tree ⁻¹)	
1. Coppice shoot regrowth (2-year study period)	221	242
2. Lower stem increment (2-year study period)	18.4	19.4
3. Root biomass increment (2-year study period)	67	78
4. Accumulated litter (6 years of trees growth)	179	192

Discussion

The $\delta^{15}\text{N}$ signals of whole-plant biomass N and of specific parts of tagasaste trees reliant solely on fixed N showed greater N isotope fractionation during partitioning of fixed N within the plant than for N_2 fixation *per se*, with nodules highly enriched, leaves and roots slightly enriched and stems significantly depleted in ^{15}N relative to atmospheric N_2 (0‰). Depletion of ^{15}N in shoots relative to nodulated roots is typical for N_2 -fixing legumes that have functional nodules enriched in ^{15}N . To date, the mechanism for this has not been elucidated. Using the $\delta^{15}\text{N}$ of leaves, stems, roots and nodules, we calculated the $\delta^{15}\text{N}$ of whole, fully symbiotic, tagasaste plants as 0.42‰ (s.e. 0.11) 145 DAS and -0.47‰ (excluding fallen leaves) 269 DAS. There are reports in the literature of fractionation due to N_2 fixation being both positive and negative for the same legume species (Shearer and Kohl 1986). The most likely reasons for whole-plant $\delta^{15}\text{N}$ of fully symbiotic legumes not approximating 0‰ are fractionations during volatile N loss or leaf fall, compounded errors in reconstruction of whole-plant $\delta^{15}\text{N}$ from component parts, contributions from seed N in large seeded species, and combined N contamination in the rooting medium. There is ample evidence that under carefully controlled conditions, rapidly growing fully-symbiotic legumes have $\delta^{15}\text{N}$ values of the entire plant approximating 0‰ (see Peoples *et al.* 1989 and discussions in Hogberg 1997 and Unkovich and Pate 2000).

The causes of significant variations in $\delta^{15}\text{N}$ between plant parts are poorly understood. In addition to nodules of legumes being enriched in ^{15}N and shoots depleted, there is a consistent trend for woody plant parts to be lower in $\delta^{15}\text{N}$ than corresponding leaf material for both legumes (Shearer and Kohl 1986; van Kessel *et al.* 1994) and non-legumes (Gebauer and Schulze 1991). We have found here that in tagasaste this occurs whether plants are dependent on N_2 fixation or are utilising both N_2 and NO_3^- . The mobilisation of N from young leafy material to later-formed woody tissue might account for this consistent difference in $\delta^{15}\text{N}$, since mobilised N is likely to be of lower $\delta^{15}\text{N}$ than its source within the leaf (Handley and Scrimgeour 1997). Notwithstanding, effects of the site of NO_3^- reduction (Pate *et al.* 1993; Evans *et al.* 1996) may still be important from the perspective of root vs shoot $\delta^{15}\text{N}$, and we interpret the higher $\delta^{15}\text{N}$ in roots (1–2.5 ‰) than shoots (0.2–2 ‰) of plants fed KNO_3^- (3.45‰) as evidence of preferential utilisation of NO_3^- in roots. The site of nitrate reduction may thus cause differences in $\delta^{15}\text{N}$ signatures between plant parts. We have not conducted a detailed study of NO_3^- reduction in tagasaste, but at our field site at Moora we failed to detect any nitrate reductase activity in leafy shoot tips or roots in a May (autumn) sampling (M. Unkovich and J. S. Pate, unpublished data) using a standard *in vivo* assay (Stewart *et al.* 1993).

The similar orders of ranking of $\delta^{15}\text{N}$ for plant components of the nitrate-treated and fully symbiotic plants, and a general increase in $\delta^{15}\text{N}$ as plants were exposed to increasing concentrations of nitrate, indicated that N isotope fractionation did not undermine ^{15}N natural abundance as a means of measuring N_2 fixation inputs in tagasaste trees. Application of the technique at our field study site at Moora also required suitable reference plants to monitor $\delta^{15}\text{N}$ of the plant-available mineral N (Pate *et al.* 1994). Shoot biomass N of shallow-rooted annual herbaceous weed species, deep-rooted perennials, xylem sap N obtained from the non-nodulated lowest regions of tap roots of tagasaste trees, and ground water itself were used to monitor possible variations in the $\delta^{15}\text{N}$ of the inorganic N available from various parts of the soil profile. The combination of broadly similar $\delta^{15}\text{N}$ (mostly within the range 3–4.5‰) for all of the above material, and the relatively low values for tagasaste coppice (-0.4 to 0.9‰), enabled reasonably reliable estimates of %Nd_{fa} using the ^{15}N natural abundance technique and the volunteer annual and perennial non-legume reference species available at the site. When making such assessments, we matched B values for whole shoot of the late harvest of pot-cultured fully symbiotic tagasaste trees against $\delta^{15}\text{N}$ of tagasaste whole coppice shoot N, and B values for leaves of the same pot-cultured plants for assessing %Nd_{fa} of the N of the sequentially collected leafy shoot tips of the alley and plantation trees.

Using these protocols, we hoped to overcome the sources of error known or likely to apply to studies of biological fixation by tree or shrub legumes using ^{15}N techniques (e.g. see discussions of Shearer *et al.* 1983; Shearer and Kohl 1986; Domenach *et al.* 1989; Danso *et al.* 1992; Ovalle *et al.* 1996; Peoples *et al.* 1996). Since the tagasaste was shown to be fixing the bulk of its N, the greatest errors with the methodology were likely to be associated with within-plant ^{15}N fractionation rather than variations in $\delta^{15}\text{N}$ of reference plants (Unkovich and Pate 2000). However, for the reasons discussed above, we do not believe that this was a serious problem in the present study.

The estimates of the amounts of N_2 fixed presented here are slightly conservative, since the analysis failed to take into account turnover of N in seasonal formation and senescence of nodule and root biomass, factors known to be of considerable magnitude in the cycling of fixed N in other shrub legumes such as *Leucaena leucocephala* (van Kessel *et al.* 1994) and in herbaceous crop and pasture legumes (see literature cited by Unkovich and Pate 2000).

The only other detailed study of N_2 fixation in tagasaste of which we are aware is that of Ovalle *et al.* (1996), who compared biomass accumulation, %Nd_{fa} and inputs of fixed N of this species to three Chilean tree legumes. Biomass production over a 2-year period from sowing was shown to be much greater in tagasaste, for which a mean %Nd_{fa} value of 86% and fixation equivalent to 49 g N tree⁻¹ year⁻¹ was

recorded. While it is clearly unwise to compare performances of the young uncut trees used in the above study directly against our own data on coppicing of much older trees, both studies suggest high levels of symbiotic competency of *C. proliferus* under the field conditions tested. Furthermore, both Ovalle *et al.* (1996) and the present study (J. S. Pate, unpublished observation) have found nodules at considerable depth (below 2 m) as well as on the upper feeding roots of field-grown tagasaste. We view the perennial character of the deeper nodules as a means of allowing fixation to continue through summer when most nodules on upper lateral roots have senesced, as has been illustrated for *Prosopis* spp. by Sprent (1987).

It is of interest to compare the values for annual inputs of fixed N made by alley and plantation trees of tagasaste at Moora with those recorded for N₂-fixing trees of other agro-ecosystems. The review of Danso *et al.* (1992) is particularly relevant in summarising data for %Nd_fa and fixation inputs for a range of tree/shrub species other than tagasaste. The values quoted are 45–65%Nd_fa and fixation inputs of 304–548 kg N ha⁻¹ year⁻¹ for various studies on *Leucaena leucocephala*, 11–18% and 43–102 kg N ha⁻¹ year⁻¹ for *Sesbania sesban*, 35–45% and 505–581 kg N ha⁻¹ year⁻¹ for *S. rostrata*, 72% and 108 kg N ha⁻¹ year⁻¹ for *Gliricidia sepium*, 60% and 94 kg N ha⁻¹ year⁻¹ for *Albizia lebbek*, and 39–76% and 43–60 kg N ha⁻¹ year⁻¹ for the *Frankia*-based symbiosis of the non-legume tree *Casuarina equisetifolia*.

While the above estimates derive mostly from radically different planting designs, management practices, tree ages, densities and climatic conditions than the present investigation, our values of 83%Nd_fa and 390 kg N ha⁻¹ year⁻¹ for plantation-grown tagasaste rank high amongst the species listed above. Moreover, the N₂ fixation performance of the species at our site in the second year after cutting (at 150–170 g N tree⁻¹) which equates to 587 kg N ha⁻¹ year⁻¹ for the plantation spacing, is at the very top of the ranges reported for the N₂-fixing species listed above, and higher than the maximal values recorded in a recent review of N₂ fixation inputs by annual crop legumes (Unkovich and Pate 2000). The value of 587 kg N ha⁻¹ year⁻¹ should be close to the maximum potentially achievable by plantation-grown tagasaste in Western Australia, since the combination of mild winters, high levels of solar radiation, and access to non-limiting supplies of non-saline ground water in addition to incident rainfall provide ideal circumstances for peak productivity and symbiotic performance in the species (Lefroy *et al.* 2001). In a later paper the N cycling reported here for tagasaste will be assessed against the N dynamics of traditional annual pasture and crop rotations and of indigenous uncleared bush in the Moora region.

Finally, it should be pointed out that while the present study shows marked benefit from tagasaste in terms of inputs of fixed N into forage biomass of agricultural ecosystems, its

limited dependence on NO₃⁻ of soil and groundwater, combined with possible additions of mineralised N from decomposition of tagasaste litter, indicate a potentially negative impact in terms of pollution of aquifers with NO₃⁻. The extent to which this occurs compared to that already occurring through use of annual legume species has still to be evaluated.

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