Nitrogen partitioning in orchard-grown *Macadamia integrifolia*

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**Summary** Nut yield is highly variable in commercial macadamia production, and to ensure that nitrogen (N) supply does not limit yield, high rates of N fertilizer are generally applied. To elucidate N source and sink relations in mature *Macadamia integrifolia* Maiden et Betche trees, we traced 15N label after injection into individual branches and, after soil application, analysed xylem sap and examined the effects of hedging on tree N relations. Xylem sap N and sugar composition and concentration changed in relation to phenology and tree management. Canopy position did not affect xylem sap N concentration but sampling date had a significant effect. Hedging in spring was associated with a rapid and dramatic reduction of the concentration of xylem sap N until the following autumn, but unhedged trees were not available to unequivocally assess the significance of the results. Following 15N-branch injection in winter, most 15N label was incorporated into flushing leaves and into bark. After 15N injection in spring, flushing leaves and flowers were most strongly 15N-labelled. In late spring, 15N label was equally incorporated by developing nuts that were retained or later abscised. Soil 15N application in summer resulted in 15N-labelling of outer and mid-canopy leaves. In the following spring, 15N label was translocated to flushing leaves, flowers and developing nuts. The results indicate that outer and mid-canopy leaves are the main N sink for soil-derived N during the reproductive phase and a N source for developing tissues during the reproductive phase. Our study provides evidence that N supply to developing nuts is not a primary cause for nut abscission, supporting the notion that high N fertilizer application rates do not improve nut retention. We propose that current orchard design and hedging practices should be reviewed in context of the role of outer canopy leaves as a source of N for reproductive tissues.

**Keywords:** amino acids, *Macadamia*, 15N-labelling, nitrogen remobilization, nitrogen storage, nut retention, xylem sap.

**Introduction** *Macadamia integrifolia* Maiden et Betche (macadamia is used hereafter) and related *Macadamia tetraphylla* Johnson are subtropical Australian rainforest trees that have been commercially cultivated for their edible kernel since the 1940s. Australia is the largest producer of macadamia kernel with a production of 41,800 tonnes in 2007 (Australian Macadamia Society http://macadamias.org/index). Premature abscission of immature fruit ~10 weeks post-anthesis results in loss of significant numbers of near full-sized nuts (Stephenson and Gallagher 1989, Trueman and Turnbull 1994). Proposed causes for the strong year-to-year yield variability include N limitation of macadamia trees (Stephenson and Cull 1986, Stephenson and Gallagher 1989). Despite the importance of N for plant growth and productivity, costs of N fertilizer and environmental concerns, there is limited knowledge about the N physiology of macadamia. Rate and timing of N application to macadamia trees was examined in relation to kernel yield, carbohydrate storage and tree N status (Stephenson and Gallagher 1989). Different N fertilizer strategies did not result in consistently higher and less variable yield (Stephenson and Gallagher 1989). High annual N fertilizer application depressed size of nuts and kernels, and although more nuts developed, these failed to fill effectively and yield was similar in trees with and without N fertilizer addition (Stephenson et al. 2000). While higher N fertilizer rates increased kernel recovery by 1.6%, yields were 17% lower at the highest N rate (Stephenson et al. 2002).

Thus, there is evidence that high N fertilizer application may not be an effective measure for increasing nut retention and yield. Despite these findings, standard industry N application rates are often substantially higher than the recommended rate of 100 kg N ha⁻¹ year⁻¹, which is based on leaf N status and crop N removal (Stephenson et al. 2000, Huett and Vimpany 2007). Nitrogen fertilization practices in bioproduction systems are increasingly scrutinized for their negative effects as off-site pollutants (Gruber and Galloway...
Identifying causes of yield variability and rationalizing N application rates are concerns of the Australian macadamia industry (Stephenson et al. 2000, 2002, Huett and Vimpany 2007). Here, we aimed to characterize N allocation and partitioning of mature macadamia trees to improve current understanding of N relations.

While deciduous trees rely almost exclusively on N stored in woody tissue for early growth in spring (Millard et al. 2006), evergreen trees store N in leaves which are potential N sources for developing tissues (Millard 1994, 1996). Compared with young trees, mature temperate trees generally have lower N uptake from soil as they rely increasingly on internal remobilization to meet N demands (Millard 1996). It is not known whether mature macadamia trees rely to a greater extent on stored N or soil N sources. It has been suggested that leaves represent a significant N storage pool in macadamia (Stephenson et al. 1986, Huett et al. 2001). Canopy management of macadamia is therefore of concern because the leaf N pool is substantially reduced by regular hedging which removes 25–50% of the outer canopy every second or third year in spring.

The amino acid composition of xylem sap is a tool for studying tree N relations, as concentration and composition of amino acids change with growth, phenology and environmental conditions (Table 1). Particular amino compounds originate from N remobilization or recent N assimilation, while others originate from both sources (Malaguti et al. 2001, Millard et al. 2006). $^{15}$N injection into tissues demonstrated that N in the vascular system is highly mobile causing rapid $^{15}$N-labelling of tissues of mature alder trees (Swanson and Myrnold 1998, Seiter and Horwath 1999). We modified existing trunk injection techniques (Horwath et al. 1992, Seiter and Horwath 1999) to approach avoids strong changes in tree-branch resource allocation and microclimate and allows examination of $^{15}$N label into vegetative and reproductive tissues. This approach avoids strong changes in tree-branch resource allocation and microclimate and allows examination of $^{15}$N label into vegetative and reproductive tissues.

### Table 1. Changes in xylem sap composition in trees in relation to environmental stimuli.

<table>
<thead>
<tr>
<th>Tree species</th>
<th>Stimulus</th>
<th>Xylem sap N compounds</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinidia deliciosa</em></td>
<td>Spring bud break and anthesis</td>
<td>GLN ↓ in structural roots and ↑ in shoots at bud break, ARG ↓ in shoots at bud break, ↑ after flowering</td>
<td>Clark and Smith 1991</td>
</tr>
<tr>
<td><em>Betula pendula</em></td>
<td>Season; N remobilization in spring</td>
<td>↑ [total N], mainly GLN, CIT</td>
<td>Millard et al. 1998</td>
</tr>
<tr>
<td><em>Camellia sinensis</em></td>
<td>Pruning</td>
<td>96% ↓ [total N] in x days</td>
<td>Selvendran 1970</td>
</tr>
<tr>
<td><em>Casuarina</em></td>
<td>Nodulation</td>
<td>↑ [total N]</td>
<td>Sellstedt and Atkins 1991</td>
</tr>
<tr>
<td><em>Citrus unshiu</em></td>
<td>$^{15}$NO$_3$ as N source, $^{15}$NH$_4^+$ as N source</td>
<td>$^{15}$N-ASN, $^{15}$N-GLN</td>
<td>Kato 1981</td>
</tr>
<tr>
<td><em>Fagus sylvatica</em></td>
<td>Internal N regulation of N uptake</td>
<td>↑ [GLN] results in ↓ NO$_3^-$ uptake from the soil</td>
<td>Collier et al. 2003</td>
</tr>
<tr>
<td><em>Malus domestica</em></td>
<td>Soil N use, N remobilization in spring</td>
<td>ASN main N compound for uptake and remobilization</td>
<td>Malaguti et al. 2001</td>
</tr>
<tr>
<td><em>Morus alba</em></td>
<td>Pruning</td>
<td>77% ↓ [total N] in 3 days</td>
<td>Suzuki and Kohno 1983</td>
</tr>
<tr>
<td><em>Olea europaea</em></td>
<td>Season</td>
<td>Mainly GLN in summer, mainly ARG autumn to spring</td>
<td>Drossoulopoulos and Navis 1988</td>
</tr>
<tr>
<td><em>Picea abies</em></td>
<td>Season</td>
<td>ARG and GLN dominant, [N] ↑ with bud break</td>
<td>Gessler et al. 1998</td>
</tr>
<tr>
<td><em>Populus × euamericana, Abies alba</em></td>
<td>Host to mistletoe N and C transfer</td>
<td>ARG ↑ with N supply exceeding tree N requirements</td>
<td>Escher et al. 2004</td>
</tr>
<tr>
<td><em>Populus trichocarpa × balsamica</em></td>
<td>N remobilization, $^{15}$N soil application</td>
<td>GLN remobilized N, $^{15}$N-labelled amides 40 days after bud break</td>
<td>Millard et al. 2006</td>
</tr>
<tr>
<td><em>Praunus avium</em></td>
<td>N remobilization, soil N use</td>
<td>GLN and ASN remobilized N, $^{15}$N-labelled amides 21 days after bud break</td>
<td>Millard et al. 2006</td>
</tr>
<tr>
<td>Tropical savanna trees</td>
<td>Season</td>
<td>ARG in dry season; ASN, GLN in wet season</td>
<td>Schmidt and Stewart 1998</td>
</tr>
</tbody>
</table>
Materials and methods

Plant material

Commercial *M. integrifolia* trees (cultivar HAES344 grafted to rootstocks of commercial variety H2) were studied. The 15-year-old trees were growing in red krasnozem of medium to fine texture without supplemental irrigation at the commercial orchard at Tregeagle (28°50′27″S 153°21′30″E), northern New South Wales, Australia. Trees were spaced 4 × 8 m with east–west row orientation at a planting density of 312 trees ha⁻¹, 6–8 m tall, with intra-row canopies forming a continuous hedgerow. Arboretum trees used in the soil ¹⁵N application experiment were grown at the nearby Alstonville Tropical Fruit Research Station, Alstonville (28°51′11″S 153°27′11″E). Mean daily maximum/minimum temperatures for winter (July) and summer (January) are 18.5/9.8°C and 27.2/19.4 °C, respectively. Average annual rainfall (1860 mm) is seasonal with maximum rainfall occurring in March (284 mm) and minimum in August (52 mm); rainfall distribution in the two sampling years is shown in Figure 3B.

Analysis of xylem sap

Eighteen trees were selected at random at the commercial orchard at Tregeagle. All experimental trees were hedged in October 2000 which removed between 0.5 and 1 m of the tree canopy from north and south orientations. Unfortunately, the trees marked to remain unhedged were accidently hedged by the operator. Xylem sap samples were collected from the northern side of the canopy during the morning (7:30–11:00). Branches from the upper (4–6 m above ground) and lower (1–2 m above ground) canopy were sampled from each tree at each sampling time. Trees were approximately sampled every 6 weeks over a 2-year period from January 2000 to November 2001. Xylem sap was collected from branches in the upper and lower canopy with a diameter of 10–15 mm. A mild vacuum of ~680 Pa was applied using a venturi effect vacuum pump (Cole Parmer model 78165-20). Bark was removed from the cut end of the branch prior to application of a vacuum to prevent contamination of xylem sap with phloem or meristematic cell content according to Bollard (1953). Sap flow was assisted by sequentially shortening the stem. Sap was collected from each branch for ~5 min and volumes collected ranged from 100 μl to 1.5 ml per branch. Sap samples were immediately placed on dry ice for transport and stored at −80 °C until analysis. Amino acids were converted to phenylthiocarbamyl derivatives according to Rosenlund (1990). A Beckman System Gold HPLC was used to quantify amino acids. Amino acids were separated using a Hypersil ODS C18 150 mm × 4.6 mm 5 μm HPLC column (Phenomenex, Australia) and a gradient modified from Vasanits and Molnar-Perl (1990). Nitrate concentration was assessed in all initial samples and selected samples throughout the measuring period following the protocol outlined in Schmidt and Stewart (1998). Nitrate levels were below detection limit (<5 nM) in all samples.

Sugars were analysed in 20-μl aliquots diluted with water to 1 ml, and 20–30 mg polyvinylpolypyrrolidone was added. Samples were shaken for 1 h at 5 °C, boiled for 5 min, cooled on ice and centrifuged (14,000 r.p.m., 10 min). One hundred microlitres of supernatant was injected into a HPLC system (Dionex DX 500, Dionex, Idstein, Germany). Separation was achieved on a CarboPac 1 separation column (2050 × 4.1 mm, Dionex) with 36 mM NaOH as eluent and a flow rate of 1 ml min⁻¹. Carbohydrates were measured with a pulsed amperometric detector equipped with an Au-working electrode (Dionex DX 500). Individual carbohydrates that eluded 8–16 min after injection were identified and quantified by internal and external standards.

Injection of ¹⁵N into branches

Nineteen trees were selected from a central row of the commercial orchard at Tregeagle. The order of trees for treatment was randomized. Individual branches were selected on four separate trees for each injection time. Branches had an approximate diameter of 25 mm and were situated 4–5 m above ground level on the northern side of the canopy to maximize sun exposure. Ammonium sulphate solution ([(¹⁵NH₄)₂SO₄, 99.7 at.% excess) was injected into one branch per tree during vegetative dormancy (May), spring flush (July), flowering (September) and early nut development (November) soon after dawn when stomatal conductance rapidly increases. To inject the ¹⁵N-labelling solution, a hole (2.5 mm diameter, 25 mm deep) was drilled at a 45° angle to the main branch axis with an electric drill (Figure 1). Debris was flushed from the hole using deionized water. A 200-μl disposable pipette tip was inserted into the hole and connected to a reservoir suspended above the injection site by silicone tubing (Figure 1). The reservoir was filled with 50 ml of 20 mM [(¹⁵NH₄)₂SO₄ solution (pH 6.0) and covered to minimize contamination and evaporation. Forty-eight hours after injection, the solution had been taken up by the branch. The syringe and tubing were removed and the hole filled using commercial wound filler. Three sub-branches situated at least 1 m above the main injection site were selected for sampling at a later date (Figure 1). Selected sub-branches were removed from injected branches at 1 week after injection to determine ¹⁵N-label incorporation into different tissues (Figure 1).

¹⁵N application to soil

Two 25-year-old trees at the Alstonville Tropical Fruit Research Station were used for the ¹⁵N application soil experiment. Nitrogen was supplied to the soil at a rate of 200 g N tree⁻¹ as [(¹⁵NH₄)₂SO₄ (2 at.% excess) in February 2001. Twenty litres of 700 mM ¹⁵N solution were distributed evenly into a 10-cm-deep trench at a radius of 1.5 m from the base of the trunk where a high abundance of fine roots was observed. The trench was immediately filled with soil, and trees were irrigated. Two branches with a diameter of 10–15 mm were collected from canopy skirt (lower canopy) and within 1.5 m of the top of the crown (upper canopy) twice weekly.
for the first 6 weeks following soil $^{15}$N application. Branches were subsequently collected at 1- and 2-week intervals for the next 7 months until November 2001.

$^{15}$N analysis of tissue

Depending on phenological stage, branches were divided into the following tissue types: woody stems, oldest leaves in the inner canopy, mature leaves in mid-canopy, youngest developed leaves in the outer canopy and developing immature leaves in the outer canopy; flower; nuts. After transfer to the laboratory, tissues were dried at 60 °C and ground to a fine powder using a ball mill. Tissues were analysed for % N and $^{15}$N abundance using a continuous flow isotope ratio mass spectrometer (Europa Tracermass, Crewe, UK) as described in Schmidt and Stewart (1999).

Statistical analysis

Statistical analysis of amino acid concentration and composition was carried out using Statistica 7.0 (StatSoft, Inc., Tulsa, OK). Data variances were normalized by addition of 1 to each value to remove errors arising from logarithmic transformation of 0 values. Data were then transformed by natural logarithm. Significance was determined by Fisher’s least significant difference or unequal N honestly significant difference post tests following analysis of variance (ANOVA). Transformations and graphing were carried out in Excel 2003 (Microsoft Corporation, USA) based on data imported from Statistica 7.0.

Significance of the incorporation and remobilization of soil-applied $^{15}$N to two tree tissues was tested by nested factorial ANOVA using Statistica 8.0 (StatSoft, Inc., Tulsa, OK). Linear regressions were calculated in GraphPad Prism v3.03 (GraphPad Software Incorporated).

Results

N content of plant tissues

Total N content was determined in tissues collected from mature macadamia trees. Young growing tissues had the highest tissue N contents ranging from 2.0 to 3.5% N in flush leaf buds and flowers, compared with mature leaves from inner (~1.0% N) and outer canopy (~1.6% N), and ~0.6% N in bark (data not shown). An overview of macadamia phenology in subtropical Australia is shown in Figure 2.

Xylem sap amino acid and sugar composition

The N composition of xylem sap was studied to determine whether recently assimilated or stored N supplies different tissues. Previous studies showed that xylem sap N composition is indicative of phenology and N relations (Table 1).
Canopy position did not affect concentration of the three main amino compounds but sampling date had a significant effect (Table 2). Nitrogen (amides and amino acids) concentration increased by 42% in late summer (February 2000) to autumn (April 2000) and decreased by 35% in winter to early spring (June to September 2000, Figure 3). Highest N concentrations in xylem sap were measured in late winter (August 2001, 2.34 ± 0.21 mM N) compared to late autumn (May 2000, 2.28 ± 0.13 mM N; Figure 3). In both years, total xylem sap N concentration decreased significantly during late September–October, from 1.44 ± 0.13 to 0.66 ± 0.08 mM N ($P < 0.0001$) and 2.04 ± 0.15 to 1.27 ± 0.09 mM N ($P < 0.01$), respectively (Figure 3).

Within 2 weeks of hedging in early October 2000, a 45% reduction in xylem sap N concentration was observed (Figure 3), but no unhedged trees were available to unequivocally establish if the reduction in xylem sap N concentration was due to hedging.

Asparagine, glutamine and arginine were the most abundant amino compounds identified in xylem sap and represented 70–95% of total xylem sap N. Concentration of these three compounds showed significant sampling time and position effects, although a significant interaction between sampling time and canopy position was only deter-

**Table 2.** Significance of time of year and canopy position for concentration of most abundant amino compounds in xylem sap of *Macadamia integrifolia* trees as determined by factorial ANOVA of ln ([amino acid] + 1).

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Time of Year</th>
<th>Canopy Position</th>
<th>Time × Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagine</td>
<td>&lt;0.0001</td>
<td>&lt;0.05</td>
<td>0.151</td>
</tr>
<tr>
<td>Glutamine</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Arginine</td>
<td>&lt;0.005</td>
<td>&lt;0.01</td>
<td>0.347</td>
</tr>
<tr>
<td>Total</td>
<td>&lt;0.0001</td>
<td>0.146</td>
<td>0.219</td>
</tr>
</tbody>
</table>

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mined for glutamine (Table 2). Asparagine concentrations in the upper canopy increased from February to April 2000 (pre-hedging), but were significantly ($P < 0.05$) lower during the same period in 2001 (Figure 4B).

Glutamine concentration in upper and lower canopy followed a seasonal pattern of accumulation and decrease prior to hedging (February–October 2000). In the year after hedging, xylem sap glutamine concentration did not continue to increase during dormancy and was significantly ($P < 0.001$) lower in late May compared with pre-hedging concentrations (Figure 4C and D). Arginine increased during vegetative dormancy and spring flush (Figure 4E and F) and significantly decreased ($P < 0.0001$) in the lower canopy in late October 2000 immediately after hedging (Figure 4F).

Concentration of three sugars measured in xylem sap showed strongest seasonal variation of sucrose with concentrations ranging from 20 to 150 mM (Figure 5A and B). Glucose concentrations changed seasonally and peaked at ~100 mM, with one sampling point at 160 mM (Figure 5C and D). Fructose concentrations were mostly around 50 mM (Figure 5E–H). After hedging, concentrations of sugars decreased, except glucose and fructose in the upper canopy. Sugar concentrations in the year following hedging were offset by ~6 weeks.

Xylem sap sucrose/N ratios in the upper and lower canopy ranged from 5 to 38 throughout the sampling period (Figure 6A and B), while combined glucose + fructose/N ratios ranged mostly between 20 and 40 (Figure 6C and D). Total measured xylem sap sugar/N ratios fluctuated in the upper and lower canopy between 50 and 75, except for the months after hedging when sugar/N ratios in the lower canopy increased to 100–200 (Figure 6E and F).

Distribution of $^{15}$N label in tissues following $^{15}$N injection into branches

All harvested tissues had measurable $^{15}$N enrichment 1 week after $^{15}$N injection into branches (Figure 7). Absolute amounts of $^{15}$N-label incorporation varied between trees and between sub-branches within a tree, but patterns of $^{15}$N incorporation were similar in sub-branches with similar pheno-ology (Figure 7). $^{15}$N incorporation occurred in flowers below one injection site during September 2000 (data not shown) indicating that injected N was also transported via the phloem towards distal tree sinks.

Young developing tissues (flush leaves, flowers and young nuts) had high $^{15}$N incorporation rates (100–1000 μg $^{15}$N g

Figure 3. Concentration of total identified amino acids in xylem sap of mature M. integrifolia trees in two consecutive years in upper and lower canopy branches (A). Trees were hedged in October 2000 (scissors symbol). Data are means ± 1 SEM for $n = 10–18$. Data were ln-transformed to normalize error and allow statistical analysis of variance. Analysis by factorial ANOVA showed that sampling date had a significant ($P < 0.0001$) effect of amino acid concentration, while canopy position ($P = 0.226$) or sampling date × canopy position ($P = 0.239$) did not (see Table 2). Daily rainfall during the sampling season (B) at the Alstonville Tropical Fruit Research Station.
dry weight\(^{-1}\) week\(^{-1}\); July, September and November). \(^{15}\)N injections during periods of rapid growth (July, September and November) resulted in up to 10-fold greater \(^{15}\)N incorporation in developing tissue than in mature tissues. \(^{15}\)N incorporation rates of flush leaves declined from July to November as leaves expanded and hardened (July 2000, September 2000 and November 2001). In macadamia trees, premature abscission of nuts occurs for ~10 weeks after anthesis (Trueeman and Turnbull 1994). Nuts that were prematurely abscised within 1 week of injection had low \(^{15}\)N incorporation rates (data not shown). Nuts prematurely abscised 7–14 days after \(^{15}\)N injection contained similar levels of \(^{15}\)N as retained nuts (November 2000). No nuts were abscised from the \(^{15}\)N-injected branches during the study period in November 2001 (Figure 7). One week after injection, abscised nuts had significantly lower \(^{15}\)N incorporation than retained nuts, while 2 weeks after \(^{15}\)N injection both groups of nuts had similar \(^{15}\)N incorporation (Figure 8).

**Incorporation of \(^{15}\)N from soil application**

We used a nested factorial ANOVA to determine change in relative \(^{15}\)N content of harvested tissues following \(^{15}\)N application to soil. Neither tree nor canopy position (upper or lower canopy) represented significant effects throughout the experiment (tree \(P = 0.942\); canopy position \(P = 0.56\)). \(^{15}\)N incorporation into outer canopy leaves (youngest mature) and mid-canopy leaves (mature) from \(^{15}\)N label applied to

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Figure 4. Seasonal changes in xylem sap concentration of upper canopy (A, C, E) and lower canopy (B, D, F) asparagine, glutamine and arginine in two consecutive years in mature *M. integrifolia* trees. Data are mean ± 1 SEM for 5–16 trees. Trees were grown in a commercial orchard in northern New South Wales, Australia, and hedged in October 2000 (see Table 2 for statistical analysis).
soil was similar and increased from 0.03 to 0.1 mg N\(^{15}\) g\(^{-1}\) within 7 weeks of \(^{15}\)N application (5 March to 9 April, \(P < 0.005\), Figure 9A). In contrast, no \(^{15}\)N incorporation was detected in old leaves (inner canopy) and bark which were statistically similar to natural abundance background of \(^{15}\)N. The rate of decrease in \(^{15}\)N label from April to July in outer and mid-canopy leaves was \(~25\%\) of the rate of increase in \(^{15}\)N incorporation seen from 5 March to 9 April (Figure 9A). From April to September 2001, specific leaf area and leaf N content did not change. Outer and mid-canopy leaves lost 50% of \(^{15}\)N during the period of 9 April to 2 July (Figure 9A). Young flush leaves showed \(^{15}\)N-label incorporation prior to increases in \(^{15}\)N content of young mature or mature leaves (March 1, Figure 9B).

**Discussion**

Nitrogen relations of orchard-grown *M. integrifolia* trees were studied to determine source/sink relations of tissues, to address the question if insufficient N supply could be a cause of premature nut abscission and to assess how hedging affects tree N relations. Evaluating the usefulness of xylem sap N and sugar analysis to assess tree N and C relations, we measured xylem sap N and sugar composition over two consecutive years and used \(^{15}\)N label to trace N uptake, remobilization and cycling in mature trees.

Sugar concentrations in macadamia xylem sap were seven- to 100-fold higher than concentrations detected in *Vitis vinifera* (Campbell and Stroher 1996), *Betula pendula* (Sauter and...
Ambrosius 1986) and Quercus robur (Heizmann et al. 2001), suggesting that macadamia trees maintain a large pool of cycling carbohydrates in the xylem compared with deciduous temperate species. In oak saplings, xylem sap delivers up to 91% of required carbohydrates to leaves, confirming the importance of xylem sap sugars for carbon supply of developing leaves (Heizmann et al. 2001), and further research has to assess the role of xylem sap sugars for developing leaves in macadamia. Prior to hedging, xylem sap sugar concentrations increased from February to April coinciding with a period of increasing trunk carbohydrates in macadamia (Huett 2004 and references cited therein) and suggesting recovery of tree carbohydrate reserves following nut maturation. We did not study the contribution of xylem sap sugars to developing tissues, but the dramatic increase in xylem sap total sugar/N ratio following hedging suggests that xylem sap N content is affected more strongly by hedging than sugar content. In perennial crops, resource status based on stored reserves is important in determining flowering intensity (McArtney and Ferree 1999). In subtropical Australia, raceme initials become visible in mid May in orchard-grown macadamia. Whether carbohydrate and N reserves in the period April–May determine the crop size in the following year remains to be investigated.

Hedging affects macadamia trees in several ways. The asynchronous flushing and flowering habit of macadamia is synchronized when pruned since time of pruning accounted for 80% of variation in flowering intensity (Olesen 2005). It is
tempting to speculate that the pronounced increase in xylem sap arginine concentration in the lower canopy over the year following hedging is caused by ongoing carbon limitation due to the concurrent carbon demand of vegetative and reproductive growth. If this is the case, subsequent N cycling and storage would likely occur as N-rich arginine rather than amides as seen in fertilized *Ligustrum ovalifolium* (Guérin et al. 2007).

An immediate and significant reduction in xylem sap amino acid N content as observed after hedging macadamia trees was also found following pruning in *Morus alba* (Suzuki and Kohno 1983) and *Camellia sinensis* (Selvendran 1970). In macadamia, post-hedging concentration of glutamine in xylem sap doubled from late spring to summer in the lower canopy. The timing of this transient increase in glutamine concentration coincided with late summer flush which can be substantial in macadamia (Stephenson and Cull 1986), especially in the lower canopy following hedging (Huet 2004). Synchronization of flushing and flowering events as a result of hedging (Olesen 2005) will result in increased N demand in lower canopy and suggests that glutamine in xylem sap is indicative of soil-derived N from recent uptake. During dormancy in winter, N concentration of xylem sap increased by ~50% suggesting an accumulation of N in the cycling N pool. This notion is supported by the rapid incorporation of $^{15}$N into outer and mid-canopy leaves following soil $^{15}$N application and subsequent export of $^{15}$N from outer and mid-canopy leaves during winter. There was a significant decrease in N concentration in xylem sap during flowering, spring flush and early nut development (August–November) that suggests that these developing tissues are strong N sinks. In autumn, mature leaves in the outer and mid-canopy were the most highly $^{15}$N-labelled tissues following soil application of $^{15}$N label in summer. From autumn to mid-winter (April to July), average $^{15}$N content decreased by 50% in outer and mid-canopy leaves, while leaf N content, specific leaf area and leaf biomass remained constant (data not shown). Reduction in $^{15}$N content in outer and mid-canopy leaves is unlikely to be due to dilution with unlabelled N, as April to July is the period of vegetative dormancy of macadamia in subtropical Australia (Stephenson and Cull 1986). Rather, the observed loss of $^{15}$N label from outer and mid-canopy leaves during this period suggests that N is redistributed to other tissues.

Mature tissues may function as a net N source for new growth or as N storage and re-allocation organs which can result in net loss of N from leaves during high N demand (Näsholm 1994, Schneider et al. 1996, Fife et al. 2008). However, similar to our results, $^{15}$N content of mature leaves of the evergreen shrub *Rhododendron ferrugineum* decreased.
without a concomitant decrease of leaf N content (Pasche et al. 2002). The authors concluded that mature *R. ferrugineum* leaves do not constitute a net N source for new growth because exported N was replaced by N derived from the vascular system (Pasche et al. 2002). Contrary to our results, a previous study found that N content of mature macadamia leaves was reduced by 25% between July and January indicating that mature leaves were N sources without being simultaneously re-supplied with N (Stephenson et al. 1986). In our study, a likely explanation for the decreased $^{15}$N levels without concomitant change in leaf N content in mature macadamia leaves is that $^{15}$N exported from these leaves is being replaced by unlabelled N from the vascular system. This indicates that outer and mid-canopy leaves form part of a mobile N pool. To confirm the generality of our results, more genotypes and growth conditions have to be studied.

Nitrogen availability affects N remobilization and N storage patterns of trees. Remobilized N supplied 44 and 10% of N required for new leaf growth when N supply was limiting or adequate in *Eucalyptus tereticornis*, respectively (Wendler et al. 1995). In high-N status *Prunus avium*, remobilization supplied 45–50% of required N to new aboveground growth, and trees had lower N uptake from soil than low-N status trees (Grassi et al. 2003). Thus, the proportion of N recycled in the xylem sap may be greater in trees with high-N status and inversely related and regulated by uptake of N from soil. Trees in our study grew in soil with high N availability (Huett and Stewart 1999) and future studies should examine macadamia leaves for similar patterns.

Figure 9. Incorporation of $^{15}$N into tissues of *M. integrifolia* following soil $^{15}$N application (200 g N as $(^{15}$NH$_4$)$_2$SO$_4$ per tree, 2 at.% excess). Label was applied in February 2001 into (A) outer (green filled triangles), mid- (green filled squares) and inner (inverted shaded triangles) canopy leaves and bark (shaded hexagons) and (B) vegetative flush (open triangles), flowers (open circles) and young nuts (shaded hexagon with cross). Data in (A) are mean ± 1 SEM for *n* = 4. This figure appears in colour in the online version of *Tree Physiology*. 

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<table>
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<tr>
<th>Date of Sampling in 2001</th>
<th>mg$^{15}$N g$^{-1}$N</th>
<th>mg$^{15}$N g$^{-1}$N</th>
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Macadamia trees under a range of N growth condition, from N-limiting to N-adequate, to elucidate the potential for N storage and remobilization.

Similar or higher $^{15}$N levels in flush leaves and flowers compared to outer and mid-canopy leaves suggest that remobilized or cycling N rather than N taken up from soil is the primary source of N for initial growth. The observed trend of decreasing $^{15}$N levels in outer and mid-canopy leaves during development of young nuts in macadamia is similar to that observed in Citrus (Legaz et al. 1995) suggesting a gradual replacement of remobilized N with soil-derived N in the developing nut crop of macadamia. Similar observations were made with deciduous Prunus avium and Populus trichocarpa $\times$ balsamifera as $^{15}$N-labelled amides, indicative of soil uptake, which increased only several weeks after bud break (Millard et al. 2006). Bark tissues of temperate and tropical trees store N in the form of proteins which are remobilized during periods of growth (Millard 1996, Schmidt and Stewart 1998, Cooke and Weih 2005). Following $^{15}$N application to soil, $^{15}$N incorporation into bark tissue was similar to inner canopy leaves and did not change significantly from onset of dormancy until spring flush. The high $^{15}$N incorporation of flushing leaves (<0.2 g fresh weight) by comparison to mature leaves and bark in macadamia is consistent with a high N demand during the cell division growth phase. Flush leaves, flowers and young nuts were highly $^{15}$N labelled following injection of $^{15}$N, but unhardened flush leaves in November had similar $^{15}$N levels to mature leaves, indicating that N was preferentially transported to the youngest leaves and reproductive tissues.

Significant premature abscission of developing nuts occurs between 8 and 10 weeks post-anthesis in macadamia and may represent up to 25% of final yield in biomass (Trueman and Turnbull 1994). $^{15}$N-labelling of nuts which were prematurely abscised 1 week after $^{15}$N injection was low, but immature nuts abscised 2 weeks after injection had similar $^{15}$N-labelling to retained nuts. This suggests that premature abscission is not linked to the N sink strength of nuts, and that initiation of abscission results in cessation of N supply to nuts between 7 and 14 days prior to nut drop. Xylem vessels within the abscission zones of leaves and fruit are structurally different to adjoining vessels above and below this zone in a wide range of plants (Andre et al. 1999). Cells within the abscission zone of peach express cell wall degrading enzymes during abscission (Bonghi et al. 2000). It is therefore possible that other xylem-supplied resources were also influenced during the week prior to nut drop, and that lower $^{15}$N incorporation into nuts abscised within 1 week of injection resulted from a general loss of connectivity with the tree prior to abscission rather than N limitation per se.

In summary, we confirm previous studies that xylem sap N and sugar concentration and composition can assist understanding of tree N relations, although more research is required before xylem sap analysis can be used as a tool for evaluating tree N status. We found evidence that N supply is an unlikely primary cause for nut abscission in macadamia because retained and abscised nuts had similar N incorporation following $^{15}$N-label injection into branches. Tracing $^{15}$N label from soil application showed that soil-derived N is initially distributed to young mature and mature leaves, which become N sources in the following spring and provide xylem-mobile N in the form of amidoxime and arginine to developing tissues. In contrast, old leaves and bark have a minor role as N sources for developing leaves, flowers and nuts as evidenced by low levels in $^{15}$N-label incorporation and loss. We conclude that N fertilizer recommendation and canopy management should be carefully reviewed to avoid unnecessary N fertilizer application.

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References


