S. Schmidt · G. R. Stewart

$\delta^{15}N$ values of tropical savanna and monsoon forest species reflect root specialisations and soil nitrogen status

Abstract A large number of herbaceous and woody plants from tropical woodland, savanna, and monsoon forest were analysed to determine the impact of environmental factors (nutrient and water availability, fire) and biological factors (microbial associations, systematics) on plant $\delta^{15}N$ values. Foliar $\delta^{15}N$ values of herbaceous and woody species were not related to growth form or phenology, but a strong relationship existed between mycorrhizal status and plant $\delta^{15}N$. In woodland and savanna, woody species with ectomycorrhizal (ECM) associations and putative N_2-fixing species with ECM/ arbuscular (AM) associations had lowest foliar $\delta^{15}N$ values (1.0–0.6‰), AM species had mostly intermediate $\delta^{15}N$ values (average +0.6‰), while non-mycorrhizal Proteaceae had highest $\delta^{15}N$ values (+2.9 to +4.1‰). Similar differences in foliar $\delta^{15}N$ were observed between AM (average 0.1 and 0.2‰) and non-mycorrhizal (average +0.8 and +0.3‰) herbaceous species in woodland and savanna. Leguminous savanna species had significantly higher leaf N contents (1.8–2.5% N) than non-fixing species (0.9–1.2% N) indicating substantial N acquisition via N_2 fixation. Monsoon forest species had similar leaf N contents (average 2.4% N) and positive $\delta^{15}N$ values (+0.9 to +2.4‰). Soil nitrification and plant NO_3^- use was substantially higher in monsoon forest than in woodland or savanna. In the studied communities, higher soil N content and nitrification rates were associated with more positive soil $\delta^{15}N$ and plant $\delta^{15}N$. In support of this notion, *Ficus*, a high NO_3^- using taxa associated with NO_3^- rich sites in the savanna, had the highest $\delta^{15}N$ values of all AM species in the savanna. $\delta^{15}N$ of xylem sap was examined as a tool for studying plant $\delta^{15}N$ relations. $\delta^{15}N$ of xylem sap varied seasonally and between differently aged *Acacia* and other savanna species. Plants from annually burnt savanna had significantly higher $\delta^{15}N$ values compared to plants from less frequently burnt savanna, suggesting that foliar ^{15}N natural abundance could be used as marker for assessing historic fire regimes. Australian woodland and savanna species had low leaf $\delta^{15}N$ and N content compared to species from equivalent African communities indicating that Australian biota are the more N depauperate. The largest differences in leaf $\delta^{15}N$ occurred between the dominant ECM Australian and African savanna (miombo) species, which were depleted and enriched in ^{15}N, respectively. While the depleted $\delta^{15}N$ of Australian ECM species are similar to those of previous reports on ECM species in natural plant communities, the ^{15}N-enriched $\delta^{15}N$ of African ECM species represent an anomaly.

Keywords ^{15}N natural abundance · Fire · Mycorrhiza · Monsoon forest · Savanna

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Introduction

Despite growing knowledge of ^{15}N natural abundance signatures in plant communities, we are yet to fully understand the causal determinants of ^{15}N natural abundance in natural systems. One difficulty is that ^{15}N fractionation occurs as a consequence of not only biological but also physico-chemical processes (Handley and Raven 1992). Among the factors influencing soil $\delta^{15}N$ values are soil age, disturbance, frequency of fire, soil moisture and rainfall, net mineralisation/nitrification potential, and point sources of isotopically distinct N (Stewart and Schmidt 1999). In previous studies of northern Australian plant communities, plant $\delta^{15}N$ values have been interpreted as being causally linked to rainfall as an inverse relationship exists between rainfall and plant $\delta^{15}N$ (Schulze et al. 1998; Austin and Sala 1999; Handley

et al. 1999). Similarly, fire (Handley et al. 1999; Cook 2001) and grazing (Schulze et al. 1999) have been suggested as potential factors influencing plant $\delta^{15}N$ values. In addition to observed trends of plant $\delta^{15}N$ across different ecosystems, strong variation in $\delta^{15}N$ may occur between species within an ecosystem (Stewart and Schmidt 1999).

Early studies of plant $\delta^{15}N$ relations suggested that $\delta^{15}N$ provides a tracer that allows tracking of plant N sources, but it has become evident that $\delta^{15}N$ of total soil N is mostly not reflected in plant $\delta^{15}N$, and due to analytical difficulties very limited information is available on the $\delta^{15}N$ of plant available soil N sources. The lack of a direct relationship between soil $\delta^{15}N$ and plant $\delta^{15}N$ values may be due to (1) plants using N forms that differ in their isotopic composition from the $\delta^{15}N$ of total soil N (Handley and Scrimgeour 1997; Högberg 1997), (2) mycorrhizal associations affecting plant $\delta^{15}N$ (Högberg et al. 1996; Schmidt and Stewart 1997; Michelsen et al. 1998; Hobbie et al. 2000), and (3) isotopic fractionation occurring during N uptake (Evans 2001; Robinson 2001; Yoneyama et al. 1991, 2001). However, while understanding of plant $\delta^{15}N$ relations in natural ecosystems is still somewhat limited, ecosystem research has demonstrated that robust patterns exist with respect to plant and soil $\delta^{15}N$ within and across ecosystems allowing the use of ^{15}N natural abundance as an integrator of N cycle processes (Robinson 2001).

Here we consider the extent to which plant $\delta^{15}N$ values reflect the influence of environmental and biological factors through an investigation of tropical woodland, savanna and monsoon forest species. These vegetation types differ in exposure and susceptibility to fire, water and nutrient status. The main taxa of Australian woody savanna species include the dominant *Eucalyptus* (Myrtaceae) with ecto- (ECM) or ecto/va-mycorrhizal (ECM/AM) associations, while most other species are va-mycorrhizal (AM). *Acacia* and other potentially N_2-fixing woody leguminous species have ECM/AM or AM associations, while Proteaceae are non-mycorrhizal but form cluster roots. Interspersed with *Eucalyptus*-dominated savannas in north Australia are pockets of fire-susceptible monsoon forests in areas characterised by higher nutrient and water availability and lower fire frequencies compared to savannas that preclude invasion from fire-tolerant savanna species (Bowman and Fensham 1991). In contrast to savannas, monsoon forests are dominated by AM species. In both communities, species differ in their N status and use of N sources (Schmidt et al. 1998; Schmidt and Stewart 1998). Seasonal differences in plant N acquisition occur because soil N appears to be taken up mostly during the wet season and internally recycled during the dry season. A strong relationship between soil and plant N status exists in these communities (Schmidt et al. 1998; Schmidt and Stewart 1998). Fire is a major factor in Australian savannas and the influence of fire on $\delta^{15}N$ relations was investigated to determine whether ^{15}N could help to reconstruct historic fire regimes. The vegetation types studied here afford a useful model system with which to investigate factors influencing plant $\delta^{15}N$ values. These plant communities were compared with corresponding African communities.

Materials and methods

Study sites and sample collections

The study was carried out in the Alligator Rivers Region of Kakadu National Park within a 30 km radius of Jabiru (12.39°S, 132.55°E) and near Darwin (12.30°S, 131.6°E, Howard River East catchment) in northern Australia. The climate is distinctly monsoonal with a long dry season (May–October) followed by a wet season (December–March) during which 90% of the average annual precipitation of 1,475 mm is received. Maximum and minimum monthly temperatures range from 18 to 25°C and 31 to 37°C in the dry and wet seasons, respectively. Sampling occurred in mid dry season (July 1994), late dry season (September 1996, 1997), early wet season (December 1995), and mid wet season (February 1995, January 1998). An annually burnt subsection (segment L) of the CSIRO Kapalga fire research site was compared with savanna that had not been burnt for 4 years at Kapalga (segment M) and one that had been burnt 2 years prior to sampling near Jabiru.

Plant species

Eucalyptus tetrodonta and *E. miniata* dominate the most distinctive savanna community in northern Australia. *Acacia* species are prominent understorey species in this open forest community. Other genera of tree and shrub species sampled in this study include *Alstonia*, *Buchanania*, *Calytrix*, *Clerodendrum*, *Cochlospermum*, *Dolichandrone*, *Erythrophleum*, *Ficus*, *Gardenia*, *Grevillea*, *Lophostemon*, *Melaleuca*, *Owenia*, *Parinari*, *Persoonia*, *Petalostigma*, *Planchonia*, *Terminalia*, and *Xanthostemon*. Soils are mostly well drained sandy loams or loams which remain temporarily water saturated during the wet season. The sandstone escarpment in Kakadu National Park supports open *Eucalyptus* woodland with sparse canopy and ground cover (Brock 1988). Escarpment species were studied at Koongarra Saddle and included many species in the same genera as listed for the savanna woodland. In addition, *Allosyncarpia*, *Bossiaea*, *Calycopeplus*, *Dampiera*, *Gompholobium*, *Maytenus*, *Myrtella*, *Pityrodia*, *Plagiocarpus*, *Solanum*, and *Vitex* were sampled. A deciduous monsoon forest near "Holiday Village" was studied and genera sampled included *Acacia*, *Adenanthera*, *Canarium*, *Cathormion*, *Cupaniopsis*, *Elaeocarpus*, *Eucalyptus*, *Exocarpus*, *Ficus*, *Ganophyllum*, *Glycosmis*, *Litsea*, *Planchonia*, *Strychnos*, *Terminalia*, *Trema*, *Vitex*, and *Wrightia*. The monsoon forest has moderately drained clayey red soil close to the flood plains. Herbaceous species in each community were sampled and included members of the following families: Araceae, Asteraceae, Commelinacae, Convolvulaceae, Curcurbitaceae, Cyperaceae, Dilleniaceae, Euphorbiaceae, Fabaceae, Goodeniaceae, Haemodoraceae, Poaceae, Liliaceae, Polygalaceae, Portulacaceae, Taccaceae, Tiliaceae, Vitaceae, and Xanthorrhoeaceae.

$\delta15N$ analysis

Healthy mature leaves, twigs and roots were oven-dried at 60°C on the day of collection. Woody plant samples were treated as described by Schmidt and Stewart (1997). Plant samples were ground to a fine powder using a ball mill (Retsch MM-2, Haan, Germany) and analysed by continuous flow isotope ratio mass spectrometry (CF-IRMS, Tracer Mass, Europa Scientific, Crewe, UK). Samples with a low N content (0.3–0.5% N) were analysed by continuous flow isotope ratio mass spectrometry (20–20 CF-IRMS Europa Scientific, Crewe, UK). Leaves, woody plant parts and twig xylem sap were sampled in the late dry season from the same tree. Xylem sap was analysed for nitrogenous compounds (Schmidt and

Stewart 1998) to calculate the amount required to obtain 20–100 g N for ^{15}N analysis. Xylem sap was freeze-dried in portions of 0.1 cm^3 to yield sufficient N, which generally required 0.6–1.5 cm^3 of sap. Bulked soil samples were taken from the upper horizon at 0–5 cm, air-dried and analysed by continuous flow mass spectrometry (see above).

Soil analysis

Mixed bed ion exchange resin (Dowex-MR3, Sigma) was used to determine the rate at which NO_3^- and $NH4^+$ were generated in the soil (Schmidt et al. 1998). Five grams of resin was placed in 5×5 cm stapled polyethylene bags (Swiss Screens PE 48GG, 365 μm mesh) and inserted horizontally in the top 5 cm of soil with minimum soil disturbance. Soil samples were collected from the top 5 cm of the soil profile and five subsamples were bulked for each soil sample. Total soil N was determined by mass spectrometry (CF-IRMS, Tracer Mass, Europa Scientific, Crewe, UK).
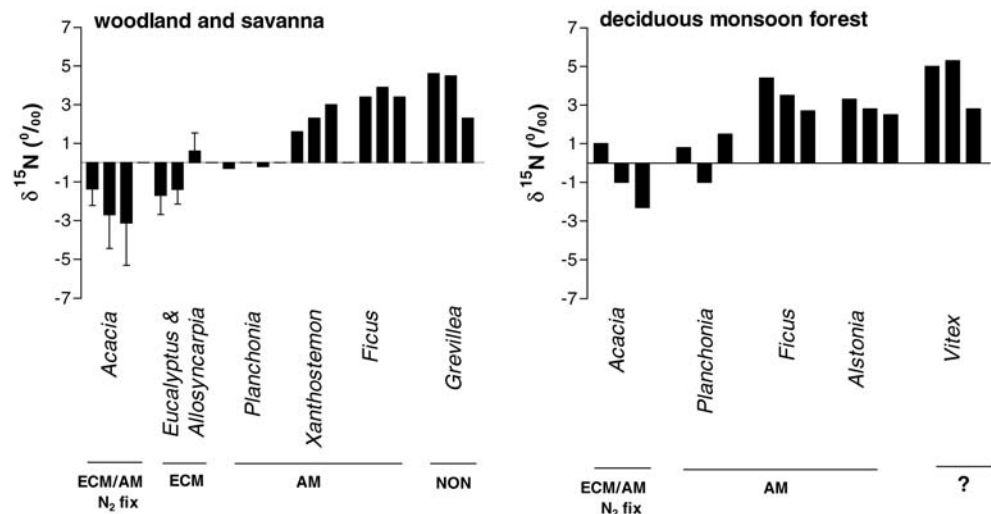
Statistical analysis

Data were analysed using STATISTICA (Statsoft, Tulsa, Oklahoma). Significant differences at the $P<0.05$ level were determined by t-test (Mann Whitney) or ANOVA (followed by Duncan's multiple range test).

Results

Intra-plant variation in $\delta^{15}N$

Previous studies have shown considerable intra-plant variation in N isotope values and in order to assess the extent of this variation in the present study, we examined representative species from three communities (Fig. 1). All parts of putative N_2-fixing species (*Acacia*, *Erythrophleum*) had negative $\delta^{15}N$ values, except leaves of a monsoon forest *Acacia* species. Leaves and twigs of ECM myrtaceous species (*Eucalyptus*, *Allosyncarpia*) had negative $\delta^{15}N$ values, while roots had positive values. AM species had variable $\delta^{15}N$ values. Non-mycorrhizal savanna species (*Grevillea*, *Banksia*) and most species in the monsoon forest had positive $\delta^{15}N$ values.

No significant seasonal variations in leaf $\delta^{15}N$ values of 23 savanna and 11 monsoon forest species were observed (data not shown), while $\delta^{15}N$ of xylem sap differed between seasons (Fig. 2). Xylem sap extracted from *Acacia*, *Eucalyptus* and *Erythrophleum* in the wet season had lower $\delta^{15}N$ values than sap collected in the dry season. Xylem sap from mature *Acacia* trees collected in the wet season had negative $\delta^{15}N$ while that of saplings and an old tree had positive $\delta^{15}N$ values. Xylem sap of AM species *Ficus* and *Planchonia* and non-mycorrhizal *Grevillea* had positive $\delta^{15}N$ values.

Foliar N content and $\delta^{15}N$ of species with different root specialisations

Herbaceous N_2-fixing species had significantly ($P<0.05$) higher leaf N contents than non-fixing AM or non-mycorrhizal species from escarpment woodland and savanna (Table 1). In the monsoon forest, foliar N content was similar in all herbaceous species. $\delta^{15}N$ values of N_2-fixing species were mostly (although not statistically significantly) lower than those of AM species, which in turn were lower than those of non-mycorrhizal species.

Acacia species had significantly ($P<0.05$) higher leaf N contents than *Eucalyptus*, AM, and proteaceous species in escarpment woodland and savanna, while similar leaf N contents were observed in all species in the monsoon forest (Table 2). While differences in leaf $\delta^{15}N$ were observed between species with different root specialisations, inspection of the data showed no relationship between $\delta^{15}N$ and phenology or growth habit in herbaceous and woody species (data not shown). In escarpment woodland and savanna, *Acacia* and *Eucalyptus* had significantly ($P<0.001$, $P<0.05$) lower $\delta^{15}N$ values than AM species and species in the Proteaceae (Table 2). Species from monsoon forest had positive $\delta^{15}N$ values.

In all communities $\delta^{15}N$ of total soil N was higher than those of woody species with mycorrhizal associations and

Fig. 1 $\delta^{15}N$ (‰) of different plant parts of species from escarpment woodland, savanna, and monsoon forest. The *three bars* per taxa represent (1) leaves, (2) twigs, (3) roots. Species in same genus with the same root specialisations were pooled. Samples for each species/genus were bulked from several leaves and branches from one tree in the dry season. Data represent averages and standard deviations. For species' details see Materials and methods

Table 1 Pooled foliar nitrogen content (%N) and $\delta^{15}N$ (‰) of herbaceous species with different root specialisations in woodland, savanna and monsoon forest communities. Averages and standard deviations are shown; n, number of species. Several individuals were bulked for each species. Significant differences ($P<0.05$, ANOVA, followed by Duncan's multiple range test) between species with different root specialisations are indicated by different letters within each community (across columns)

	N_2-fixing AM species	n	AM species	n	Non-mycorrhizal species	n
Foliar N content (% N) of herbaceous species						
Escarpment woodland	2.9 (0.1)[a]	2	1.0 (0.4)[b]	9	0.9 (0.5)[b]	3 $P<0.001$
Savanna	2.3 (0.6)[a]	6	1.3 (0.8)[b]	17	1.0 [b]	2 $P<0.05$
Annually burnt savanna	2.6 (0.6)[a]	5	1.1 (0.7)[b]	6	0.7 [b]	1 $P<0.05$
Monsoon forest	3.2 (0.3)	6	3.2 (0.7)	12	–	
Foliar $\delta^{15}N$ (‰) of herbaceous species						
Escarpment woodland	−0.2	2	−0.1 (1.4)	9	+0.8 (1.8)	3
Savanna	−1.1 (0.6)	6	−0.2 (1.3)	17	+0.3	2
Annually burnt savanna	−0.5 (0.8)	5	+1.0 (1.0)	5	+1.8	1
Monsoon forest	+0.3 (0.6)[a]	6	+2.3 (0.7)[b]	12	-	$P<0.001$

Table 2 Foliar $\delta^{15}N$ (‰) values and leaf N content (%N) of woody species from woodland, savanna, and monsoon forest with different root specialisations. *Acacia* spp. mostly ECM/AM and putative N_2-fixing; *Eucalyptus* spp. and *Allosyncarpia* ECM with some *Eucalyptus* spp. ECM/AM; *Grevillea, Persoonia* (Proteaceae) non-mycorrhizal. AM species include all va-mycorrhizal and non-fixing species. Averages and standard deviations are shown; n, number of species (number of samples in parentheses). Different letters within columns indicate significant differences between groups (ANOVA, followed by Duncan's multiple range test) within each plant community. Nitrogen content (% N) and $\delta^{15}N$ of total soil originating from the top 5 cm is shown for each community

	Root specialisation	N (%)	$\delta^{15}N$ (‰)	Range (‰)	n
Escarpment woodland					
Acacia	ECM/AM, N_2-fix	1.8 (0.6)[a]	−0.1 (0.4)[a]	−1.3 to −0.5	4 (4)
Eucalyptus, Allosyncarpia	ECM(AM)	1.1 (0.3)[b]	−0.6 (0.3)[a]	−1.3 to −0.6	3 (3)
AM species	AM	1.1 (0.3)[b]	+0.6 (1.1)[b]	−1.3 to +2.2	14 (14)
Grevillea/Persoonia	Non-mycorrhizal	0.9 (0.3)[b] ($P<0.05$)	+4.1 (1.1)[c] ($P<0.05$)	+2.9 to +5.0	3 (3)
	Total soil N	0.11 (0.04)	+2.0 (1.0)		
Savanna					
Acacia	ECM/AM, N_2-fix	2.5 (0.6)[a]	−0.7 (0.4)[a]	−1.3 to −0.2	4 (7)
Eucalyptus	ECM(AM)	1.1 (0.4)[b]	−0.6 (0.6)[a]	−1.6 to +0.3	4 (11)
AM species	AM	1.2 (0.6)[b]	+0.5 (0.7)[c]	−0.5 to +3.9	20 (35)
Grevillea/Persoonia	Non-mycorrhizal	1.0 (0.3)[b] ($P<0.001$)	+2.9 (1.0)[b] ($P<0.001$)	+1.2 to +4.5	4 (8)
	Total soil N	0.12 (0.1)	+2.5 (1.0)		
Monsoon forest					
Acacia	ECM/AM, N_2-fix	2.4	+1.0	+0.4 to +1.5	1 (2)
Eucalyptus	ECM(AM)*	1.3 (0.3)	+1.7 (0.8)	+0.7 to +2.2	1 (3)
AM species	AM	2.4 (0.9)	+2.4 (1.1)	+0.3 to +4.5	17 (22)
	Total soil N	0.43 (0.1)	+4.7 (0.1)		

* ECM status of *Eucalyptus alba*, the only *Eucalyptus* species sampled in the monsoon forest was not determined. Mycorrhizal associations were investigated as part of the mining rehabilitation research (Supervising Scientist for the Alligator Rivers Region). This database of approximately 250 species and Reddell and Milnes (1992) were used to assign species mycorrhizal associations

putative N_2-fixing species with mycorrhizal associations (Table 2). Only non-mycorrhizal Proteaceae had more positive average $\delta^{15}N$ values than $\delta^{15}N$ of total soil N of escarpment woodland and savanna.

Effects of fire regime and soil N relations on plant $\delta^{15}N$ values

Comparison of pooled foliar $\delta^{15}N$ values of fifteen herbaceous and woody species from annually burnt and less frequently burnt (≥ 2 year burning intervals) savanna sites indicated that annual burning was associated with increased foliar $\delta^{15}N$ values ($P<0.05$) (Fig. 3). Similarly, total soil $\delta^{15}N$ was higher (3.2‰) in annually burnt savanna compared with infrequently burnt savanna (2.5‰) (Fig. 3).

Five species from savanna and monsoon forest had significantly ($P<0.05$) higher $\delta^{15}N$ values when growing in monsoon forest compared to savanna (Fig. 3) whereas *Exocarpos latifolius*, a root hemi-parasite, had similar positive $\delta^{15}N$ values in both communities.

Plant available NH_4^+ and NO_3^- levels determined using in situ ion exchange resin bags showed that the proportion of NO_3^- relative to NH_4^+ was higher in soil from monsoon forest than soils from woodland and
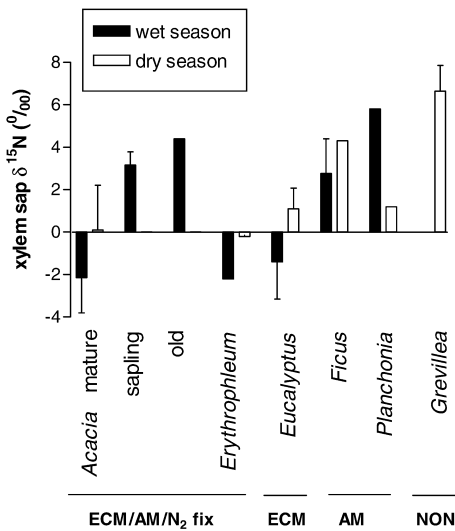
Fig. 2 Xylem sap $\delta^{15}N$ of savanna woodland species collected in wet and dry season. Unless otherwise stated, samples were taken from mature individuals. The old *Acacia* tree had no new growth and sparse foliage, saplings were approximately 2 m tall. Each value represents bulked xylem sap from several twigs of one tree. Standard deviations are shown where more than two trees were sampled
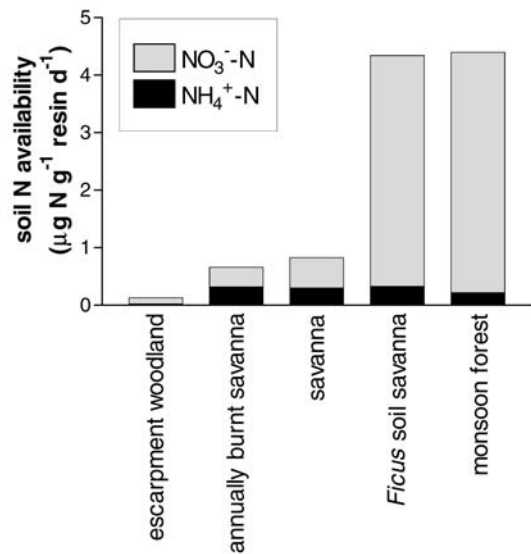

Fig. 4 Plant available soil N as determined with in situ ion exchange resin bags in the upper 5 cm of soil. Data represent averages from dry and wet season for each of the studied communities and from soil associated with *Ficus* in savanna (adapted from Schmidt et al. 1998)

Comparison of foliar N content and $\delta^{15}N$ of Australian and African species
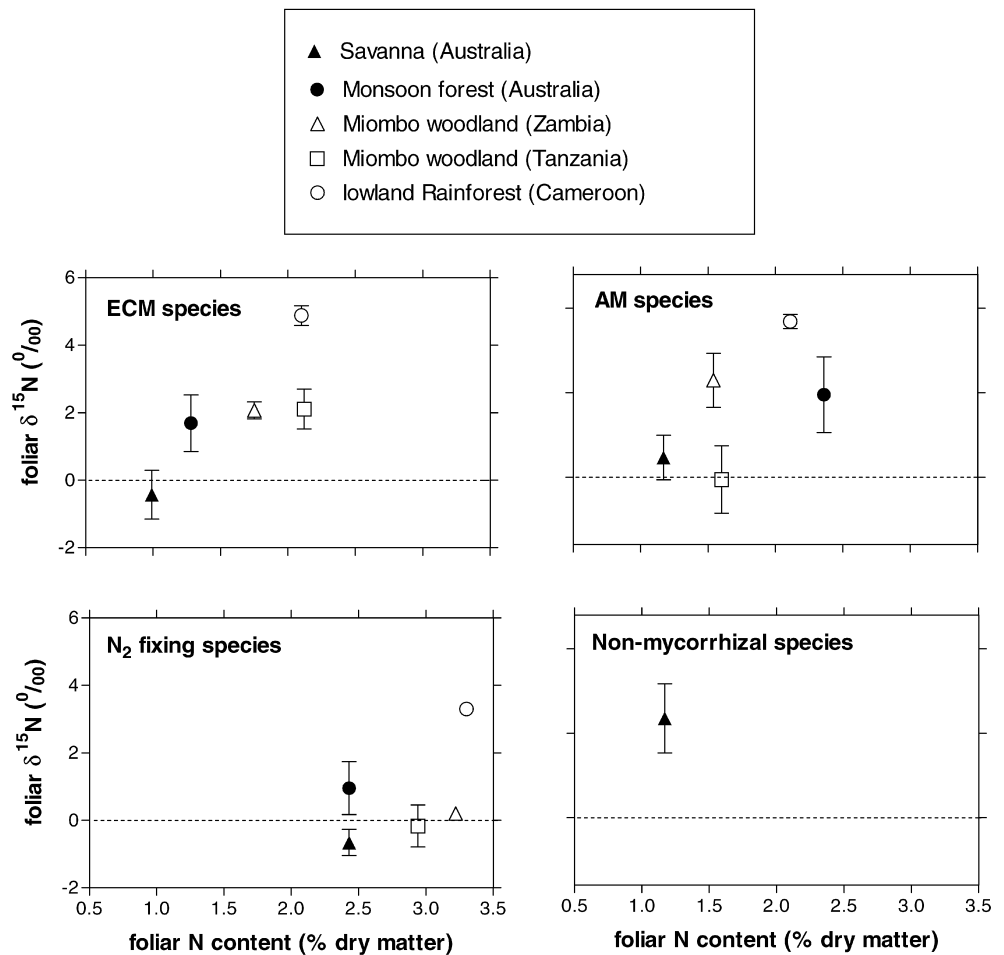
ECM, AM and N_2-fixing species from Australian savanna had lower average foliar N contents than equivalent species from African miombo communities (Fig. 5). Similarly, ECM and N_2-fixing species from Australian monsoon forest had a lower average leaf N content than their African counterparts. Only AM species in Australian monsoon forest had somewhat higher leaf N content than African rainforest species. Leaf $\delta^{15}N$ values of ECM savanna species were low compared to ECM miombo species, while N_2-fixing savanna and miombo species had similar $\delta^{15}N$ values. Average $\delta^{15}N$ of AM species differed between Zambian and Tanzanian miombo communities (+2.8 and 0.1‰) and Australian AM savanna species had intermediate $\delta^{15}N$ values. In African rainforest, N_2-fixing, AM, and ECM species had more positive $\delta^{15}N$ values than species in Australian monsoon forest.

Discussion

Plant $\delta^{15}N$ values, mycorrhizal associations and N sources

Analysis of plants in tropical woodland, savanna and monsoon forest communities revealed that plants with different root specialisations have distinct $\delta^{15}N$ values. In woodland and savanna, leaves of ECM species and to a lesser extent AM species had lower $\delta^{15}N$ than those of non-mycorrhizal species confirming the notion that presence of ECM and AM associations is associated with isotopic depletion of the host (Stock et al. 1995; Högberg

Fig. 3 Comparison of $\delta^{15}N$ (‰) of species growing at infrequently and annually burnt savanna sites ($n=15$), and of species growing at savanna and monsoon forest ($n=6$). Averages±SEM are shown. Statistical differences ($P<0.05$) between species from two sites are indicated by different letters. *Asterisk* indicates total soil $\delta^{15}N$ in each community

savanna (51–53% NO_3^- of total available inorganic N) (Fig. 4). Similar levels of NH_4^+ and NO_3^- were measured in savanna soil at annually and less frequently burnt savanna, although total soil N content was reduced in annually burnt savanna (0.06±0.01% N) compared with less frequently burnt savanna (0.12±0.01% N). Soil associated with *Ficus* in savanna had a similar NO_3^- availability as soil from monsoon forest (Fig. 4).

et al. 1996; Schmidt and Stewart 1997; Michelsen et al. 1998; Hobbie et al. 2000). Positive δ^{15}N values of roots compared to negative δ^{15}N values of stems and leaves in ECM savanna species support the view that discrimination against ^{15}N occurs during fungal N assimilation, which results in ^{15}N enrichment in fungal tissue associated with fine roots, while host tissue such as leaves or stems becomes depleted in ^{15}N relative to fungal tissue (Högberg et al. 1996; Schmidt and Stewart 1997).

Woody AM species in woodland and savanna had mostly higher δ^{15}N values than ECM(AM) and N$_2$-fixing species, similar to previous studies where AM species consistently had higher δ^{15}N values than species with ecto or ericoid mycorrhizal associations (Pate et al. 1993; Stock et al. 1995; Nadelhoffer et al. 1996; Schmidt and Stewart 1997; Michelsen et al. 1998). In our study the spread of δ^{15}N values was greater in AM species than in any other group, ranging from negative δ^{15}N values similar to those of ECM species to the isotopically enriched values that characterised non-mycorrhizal species. Woody AM species contained the most diverse taxa of the groups studied here, and the range of δ^{15}N in AM species could reflect this heterogeneity. In addition to the possible effect of mycorrhizal fungal associations on plant δ^{15}N values, soil N sources, soil N concentration and plant

N assimilation characteristics influence plant δ^{15}N (reviewed by Evans 2001; Robinson 2001). Low plant δ^{15}N values may indicate that soil N sources are used which require access and assimilation via mycorrhizal associations, while N sources that are taken up and assimilated directly by the plant may result in higher plant δ^{15}N values (Evans 2001). Some AM species had a higher NO_3^- use than the majority of savanna species and the most conspicuous among these high NO_3^--assimilating physiotypes are *Ficus* species (Schmidt et al. 1998). Interestingly, *Ficus* also had the highest δ^{15}N values of all AM species. Similarly, the herb *Ptilotus* had the highest NO_3^- use and highest δ^{15}N values of species in *Banksia* woodland (Pate et al. 1993). Nitrate reductase activity was high in leaves of *Ficus* and *Ptilotus* (Pate et al. 1993; Schmidt et al. 1998). It has been suggested that NO_3^- reduction in leaves results in little discrimination against ^{15}N while NO_3^- reduction in the roots causes efflux of a ^{15}N-enriched N fraction and plant ^{15}N depletion relative to the source (Evans 2001). While intra-plant isotopic fractionation may affect annual species (Evans 2001), *Ficus* leaves, stems and roots were similarly enriched in ^{15}N indicating little intra-plant isotopic fractionation. A possible explanation for high δ^{15}N values of high NO_3^- using species is the observation that plants discriminate

more strongly against $^{15}NH_4^+$ than against $^{15}NO_3^-$ in hydroponic culture (Mariotti et al. 1980; Yoneyama et al. 1991, 2001). If stronger isotopic discrimination occurs with NH_4^+ as a N source compared to NO_3^- as a N source, preferential use of NO_3^- may contribute to the observed differences in $\delta^{15}N$ values between species with high NO_3^- use such as *Ficus* and AM species with a low NO_3^- use. Lastly, differences in $\delta^{15}N$ between plants with high or low NO_3^- use could be caused if soil NO_3^- was ^{15}N enriched compared to soil NH_4^+, for example if ^{15}N depleted NO_3^- is lost from the soil through denitrification (Högberg 1997).

The high $\delta^{15}N$ of monsoon forest soil, the greater N content and availability of NO_3^- compared to savanna and woodland soil, supports the notion that stronger mineralisation and nitrification result in more positive $\delta^{15}N$ of soil N fractions (Handley and Raven 1992). Monsoon forest plants had higher $\delta^{15}N$ values and a greater NO_3^- use compared to the majority of plants in woodland and savanna (Schmidt et al. 1998). Tree species growing at both savanna and monsoon forest had significantly ($P<0.05$) higher $\delta^{15}N$ values when growing in the monsoon forest (average 2.8±0.3‰) compared to savanna (average 0.7±0.8‰). The high $\delta^{15}N$ values of plants growing at monsoon forest compared to savanna may be caused by (1) high $\delta^{15}N$ of soil N sources as a result of high N turnover, nitrification and/or N loss, (2) high use of NO_3^- and associated lower fractionation against ^{15}N compared to NH_4^+ use, (3) low contribution of mycorrhizal fungi to plant N acquisition and associated lower discrimination against ^{15}N during fungal N transfer.

Plant $\delta^{15}N$ and N_2 fixation

Leaf $\delta^{15}N$ values of putative N_2-fixing species were in the range often associated with N_2 fixation, but were indistinguishable from $\delta^{15}N$ of myrtaceous ECM species in woodland and savanna. Since putative N_2-fixing *Acacia* species have ECM and AM associations, it is not possible to separate effects of N_2 fixation and mycorrhizal associations on plant $\delta^{15}N$. However, the high leaf N content of *Acacia* and other putative N_2-fixing species in comparison to non-fixing species in savanna and woodland suggests that a substantial proportion of plant N is derived from N_2 fixation. Leguminous species (*Bossiaea*, *Cajanus*, *Galactia*, *Gompholobium*, *Plagiocarpus*) with AM associations from woodland and savanna had low $\delta^{15}N$ and high leaf N contents indicating that the combination of AM and putative N_2 fixation results in $\delta^{15}N$ values similar to those of ECM/AM *Acacia* species. A previous study found large variations between $\delta^{15}N$ of N_2-fixing and non-fixing savanna species along an aridity gradient (Schulze et al. 1991) on which calculations of the contribution of N_2 fixation to leaf N were based. However, the recent insights that an inverse relationship exists between rainfall and plant $\delta^{15}N$, and that mycorrhizal associations, among other factors, affect plant $\delta^{15}N$ values prevents straightforward calculations of N_2 fixation rates in savannas and other plant communities.

A strong difference existed in xylem sap $\delta^{15}N$ of mature *Acacia* trees and adjacent young saplings and old tree in the wet season, since xylem sap $\delta^{15}N$ of saplings and old tree was 5–6.3‰ higher than xylem sap of mature *Acacia*. The observed differences in xylem sap $\delta^{15}N$ between differently aged *Acacia* may point to differences in N_2 fixation rates and/or N assimilation characteristics associated with maturing and ageing of *Acacia*, and this finding needs to be explored further in combination with measurements of actinorhizal and mycorrhizal associations. Similar seasonal differences in xylem sap $\delta^{15}N$ as those observed in mature *Acacia* were also observed in the putative N_2-fixing species *Erythrophleum chlorostachys* and in non-fixing *Eucalyptus*. Xylem sap $\delta^{15}N$ may reflect seasonal differences in plant N assimilation and transport, and it has been suggested that savanna trees rely soil N sources during the wet season when soil N availability is high, whereas trees remobilise and transport internally stored N sources during the dry season (Schmidt et al. 1998; Schmidt and Stewart 1998).

Plant $\delta^{15}N$ and burning regimes

Annual burning of savanna resulted in a trend of soil and leaf ^{15}N enrichment compared to less frequently burnt savanna, which contrasts with Cook's (2001) study at nearby sites. Total soil N content was lower in annually burnt savanna than in less regularly burnt savanna in our study (Schmidt et al. 1998) and in a previous study (Mordelet et al. 1996), while Cook (2001) found no difference in total soil N content of sites with different fire histories. Although is has previously been suggested that annual burning of savannas does not result in net N loss from the ecosystem (Holt and Coventry 1990), Cook (1994) estimated that annual burning causes net N losses of 1.5–2 g N m^{-2} year^{-1}. It is currently of considerable interest to devise burning regimes for sustainable management of Australia's tropical savannas. Nitrogen loss from temperate plant communities resulted in ^{15}N enrichment of the remaining soil N fractions (Högberg 1997). $\delta^{15}N$ values from savanna with different fire frequencies and analysis of historic samples could be used to address the question of how naturally occurring fires and traditional Aboriginal burning practices have influenced savanna N relations in the past.

Comparing $\delta^{15}N$ of Australian and African plant communities

^{15}N natural abundance studies in African plant communities first indicated that mycorrhizal associations could affect plant $\delta^{15}N$ values (Högberg 1990; Högberg and Alexander 1995). In order to understand plant $\delta^{15}N$ values in relation to African plant communities, $\delta^{15}N$ and leaf N

content of Australian savanna and monsoon forest species were compared with equivalent species from African miombo and rainforest. Similar to Australian savanna, African miombo woodland is dominated by non-nodulated species with ECM associations (Högberg 1990). Similarities exist in vegetation structure and nutrient relations between African and Australian communities (Mott et al. 1985). However, African miombo species had consistently higher leaf N contents than equivalent species with similar root specialisations in Australian savanna. The overall lower leaf N content of Australian savanna species compared to African species supports the notion that Australian savanna and woodland biota are the more N depauperate, which can be linked to the low soil N status of these ecosystems.

There were consistent differences between $\delta^{15}N$ values of African and Australian plants communities with African species having the more positive $\delta^{15}N$ values; the only exception being AM species in Tanzanian woodland which had similar $\delta^{15}N$ values as Australian savanna species. The suggested "$\delta^{15}N$ lowering effect" of EC mycorrhizal associations on plant $\delta^{15}N$ in Australian woodland and savanna contradicts the isotopic enrichment of African ECM species. Indeed Högberg's studies (Högberg 1990; Högberg and Alexander 1995) appear to be the only examples of ^{15}N enrichment of ECM species relative to species with other root specialisations.

Differences in plant $\delta^{15}N$ values across ecosystems in relation to soil N status and root specialisations indicate that ^{15}N natural abundance is a marker for ecosystem N relations, however, but further knowledge is required to understand the interactions of soil-plant ^{15}N natural abundance at the ecosystem scale. It would be particularly interesting to investigate how $\delta^{15}N$ of soil NH_4^+ and NO_3^- relate to $\delta^{15}N$ of xylem sap in species with different mycorrhizal associations and N assimilation characteristics.

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