

Natural abundance ($\delta^{15}\text{N}$) indicates shifts in nitrogen relations of woody taxa along a savanna–woodland continental rainfall gradient

Fiona M. Soper · Anna E. Richards · Ilyas Siddique ·
Marcos P. M. Aidar · Garry D. Cook ·
Lindsay B. Hutley · Nicole Robinson · Susanne Schmidt

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Abstract Water and nitrogen (N) interact to influence soil N cycling and plant N acquisition. We studied indices of soil N availability and acquisition by woody plant taxa with distinct nutritional specialisations along a north Australian rainfall gradient from monsoonal savanna (1,600–1,300 mm annual rainfall) to semi-arid woodland (600–250 mm). Aridity resulted in increased ‘openness’ of N cycling, indicated by increasing $\delta^{15}\text{N}_{\text{soil}}$ and nitrate:ammonium ratios, as

plant communities transitioned from N to water limitation. In this context, we tested the hypothesis that $\delta^{15}\text{N}_{\text{root xylem sap}}$ provides a more direct measure of plant N acquisition than $\delta^{15}\text{N}_{\text{foliage}}$. We found highly variable offsets between $\delta^{15}\text{N}_{\text{foliage}}$ and $\delta^{15}\text{N}_{\text{root xylem sap}}$, both between taxa at a single site (1.3–3.4 ‰) and within taxa across sites (0.8–3.4 ‰). As a result, $\delta^{15}\text{N}_{\text{foliage}}$ overlapped between N-fixing *Acacia* and non-fixing *Eucalyptus/Corymbia* and could not be used to reliably identify biological N fixation (BNF). However, *Acacia* $\delta^{15}\text{N}_{\text{root xylem sap}}$ indicated a decline in BNF with aridity corroborated by absence of root nodules and increasing xylem sap nitrate concentrations and consistent with shifting resource limitation. *Acacia* dominance at arid sites may be attributed to flexibility in N acquisition rather than BNF capacity. $\delta^{15}\text{N}_{\text{root xylem sap}}$ showed no evidence of shifting N acquisition in non-mycorrhizal *HakealGrevillea* and indicated only minor shifts in *Eucalyptus/Corymbia* consistent with enrichment of $\delta^{15}\text{N}_{\text{soil}}$ and/or decreasing mycorrhizal colonisation with aridity. We propose that $\delta^{15}\text{N}_{\text{root xylem sap}}$ is a more direct indicator of N source than $\delta^{15}\text{N}_{\text{foliage}}$, with calibration required before it could be applied to quantify BNF.

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F. M. Soper (✉)
Department of Ecology and Evolutionary Biology,
Cornell University, Ithaca, NY 14853, USA
e-mail: fms46@cornell.edu

A. E. Richards · G. D. Cook
CSIRO Ecosystem Sciences, PMB 44, Winnellie,
NT 0822, Australia

I. Siddique
Department of Crop Science, Universidade Federal de Santa
Catarina, Florianópolis, SC 88034001, Brazil

M. P. M. Aidar
Institute of Botany, SMA, PB 68041, São Paulo CEP 04045-972,
Brazil

L. B. Hutley
School of Environment, Research Institute for the Environment
and Livelihoods, Charles Darwin University, Darwin, NT 0909,
Australia

N. Robinson · S. Schmidt
School of Agriculture and Food Sciences, The University
of Queensland, Brisbane, QLD 4072, Australia

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Introduction

Interactions between soil nutrient availability and plant nutrient acquisition strategies are drivers of ecosystem structure, function and productivity (Grime 2002; Hobbie 1992; Knops et al. 2002; Lavorel and Garnier 2002). In tropical savannas and woodlands, both water and nitrogen (N) are believed to constrain productivity (Kanniah et al. 2011). However, the relationship between water

availability, soil nutrient availability and plant N acquisition strategies remains poorly understood. This relationship is relevant given that savannas and woodlands cover over ~40 % of the tropics and are predicted to experience changing precipitation patterns over the coming century (Hughes 2003; Scholes and Archer 1997; Whitehead et al. 2000). Water availability may modulate plant N acquisition through several mechanisms. Directly, water availability affects potential for biological N fixation (BNF; via viability of rhizobial symbionts or carbon supply to nodules, Hartwig 1998) and influences plant growth and thus N demand. Indirectly, water influences N availability by affecting N movement in soil (Nye and Tinker 1977), rates of mineralisation, ammonification and nitrification (Pastor and Post 1986) and gaseous N losses (Hutchinson et al. 1993; Skiba and Smith 2000). However, interpretation of N acquisition by woody plants is often hampered by methodological limitations, particularly in the case of BNF, which is notoriously challenging to measure (Cleveland et al. 2010; Galloway et al. 2008; Vitousek et al. 2002).

The N isotopic ratio ($\delta^{15}\text{N}$) of soil provides an integrated measure of relative rates of N inputs and losses (Handley and Raven 1992; Nadelhoffer and Fry 1994), parameters that can be strongly dependent on abiotic conditions (Amundson et al. 2003; Högberg 1997). Inputs to natural ecosystems typically come from BNF (~0 ‰; Shearer and Kohl 1986) or from atmospheric deposition (generally cited as -3 to 3 ‰ but sometimes lower; Elliott et al. 2007; Freyer et al. 1996; Handley et al. 1999; Houlton et al. 2006), while losses derive from gas flux (with an enriching fractionation of 28–60 ‰; Robinson 2001) or leaching (with a typically minor fractionation effect; Handley et al. 2001; Houlton et al. 2006). Ecosystems that recycle proportionally more N through the plant uptake–decomposition–mineralisation and/or nitrification process will experience less enrichment of the soil pool than ‘leakier’ ecosystems in which more N is lost (via gas loss or leaching) relative to turnover. As such, soil N cycling can be thought of as laying on a continuum from more ‘closed’ (relatively higher rates of recycling) to more ‘open’ (relatively higher rates of fractionating N losses; Austin and Vitousek 1998). Factors that promote losses, such as reduced plant N demand due to low gross primary productivity (GPP), limitation by another nutrient, limitation by water (Austin and Vitousek 1998) or pulsed rain events which decouple plant N demand and supply (Austin et al. 2004) lead N to be less tightly conserved. This interpretation is supported by observations of an inverse relationship between soil $\delta^{15}\text{N}$ and precipitation across a wide range of ecosystems (Amundson et al. 2003; Austin and Vitousek 1998; Aranibar et al. 2004; Handley et al. 1999; McCulley et al. 2009). N cycle openness is therefore a useful concept for identifying limitations to ecosystem productivity.

The North Australian Tropical Transect (NATT) is a 1,000-km north–south continental rainfall gradient (1,600–250 mm mean annual precipitation) from woodland to savanna dominated by woody taxa with distinct N acquisition strategies. The transect offers a unique opportunity to explore how N acquisition changes over large scales within and between taxa in relation to water and N availability. Along the NATT, water availability becomes more temporally heterogeneous (isolated storms rather than continuous monsoon) as average soil moisture content decreases and water deficit increases (Eamus and Prior 2001), resulting in a decline in GPP and biomass from north to south (Cook et al. 2002; Hutley et al. 2011; Kanniah et al. 2011). We propose that these patterns have several consequences for N availability and acquisition. Specifically, we hypothesise that declining rainfall is associated with reduced rates of BNF, a change in proportion of inorganic N forms, a shift from N to water limitation, and decoupling of plant N demand and N supply that results in a more ‘open’ N cycle and isotopic enrichment of soil N. This paradigm of water–N relationships is supported by observations along the Kalahari transect, a climatically comparable rainfall gradient in semi-arid southern Africa (Aranibar et al. 2004; Feral et al. 2003; Swap et al. 2004), and we propose that major drivers may be common to both systems.

Woody plant taxa present along the NATT display distinct nutritional strategies. *Acacia* species (Fabaceae) commonly form N-fixing rhizobial symbioses that can supply up to 70 % of the plant N budget (Guinto et al. 2000; Schulze et al. 1991) and have arbuscular and/or ecto-mycorrhizal symbioses (Sprent 1995). *Eucalyptus* and *Corymbia* species (Myrteaceae) also form arbuscular and/or ecto-mycorrhizal symbioses (Eckhard et al. 1995; Ferrol and Pérez-Tienda 2009; Newman and Reddell 1987), while species in genera *Grevillea* and *Hakea* (Proteaceae) are non-mycorrhizal but form cluster roots (Dinkelaker et al. 1995). From an ecosystem perspective, BNF by *Acacia* species is likely to be the main N input. *Acacia* plantations in the region fix up to 12 kg N ha⁻¹ year⁻¹ (Langkamp et al. 1979), significant given that atmospheric N deposition is low (1–2 kg N ha⁻¹ year⁻¹; Galloway et al. 2008) and losses from frequent burning in monsoonal savannas approach 24 kg N ha⁻¹ year⁻¹ (Cook 1994; Rossiter-Rachor et al. 2008). Although the ability to nodulate is universal among Australian *Acacia* species (Sprent 2005), BNF may be limited by environmental conditions. *Acacia aneura* (mulga) is a dominant species in semi-arid regions and fixes N in oligotrophic sand dunes but not in typical mulga soils that have comparatively high levels of soil N (Erskine et al. 1996; Pate et al. 1998). Similarly, other Australian *Acacia* species have low rates of BNF under high temperatures and limiting water availability (Hansen and Pate 1987; Lawrie 1981).

In situ quantification of BNF in woody taxa remains a methodological challenge (Cleveland et al. 2010; Galloway et al. 2004). Acetylene reduction as a proxy for nitrogenase activity is debated and nodule recovery from deep-rooted woody perennials is difficult (Boddey et al. 2000; Hartley et al. 2007; Minchin et al. 1986). Foliar $\delta^{15}\text{N}$ is widely used to estimate the proportion of N obtained from BNF based on the premise that leaves reflect the isotopic composition of the N source; $\sim 0\text{‰}$ for BNF or typically enriched for soil-derived sources (Boddey et al. 2000; Högberg 1997; Shearer and Kohl 1986). However in practice, foliar $\delta^{15}\text{N}$ yields equivocal results because values for N-fixing and non-fixing species frequently overlap (Högberg 1997; Pate et al. 1993; Schmidt and Stewart 2003).

Numerous poorly quantified processes contribute to the $\delta^{15}\text{N}$ of plant tissues along the pathway from soil uptake to foliar integration (Evans 2001a, b). Isotopic fractionation may occur when N is taken up from soil (potentially mediated by mycorrhizae; Hobbie and Högberg 2012) and assimilated prior to xylem loading (Robinson et al. 1998; Yoneyama 1995). In leaves, $\delta^{15}\text{N}$ is further affected by rates of tissue assimilation and N turnover, leaf age, rates of photorespiration and other processes, all likely to differ between taxa (Evans 2001a, b; Kolb and Evans 2002; Liu et al. 2013; Tcherkez and Hodges 2008). These fractionation effects are likely to be increasingly compounded along the uptake and integration pathways of N. Thus, we propose that $\delta^{15}\text{N}$ in xylem sap, rather than foliage, may better represent the isotopic ratio of acquired N and facilitate more direct comparison between taxa. Fractionation prior to xylem loading may have a comparatively small isotopic effect compared with BNF, so that xylem sap could be considered a semi-qualitative indicator of BNF. This is supported by observations that $\delta^{15}\text{N}_{\text{xylem sap}}$ was lower in actively N-fixing soybean plants and *Acacia* trees than in non-nodulating soybean or *Acacia* saplings and moribund trees (Yoneyama et al. 2000; Schmidt and Stewart 2003).

Here, we assess soil N availability and compare N relations of three major woody taxa with distinct root specialisations along a continental rainfall gradient. We test the hypotheses that (1) soil N availability and cycling change with increasing aridity, (2) this change is correlated with shifts in plant N acquisition, including a decrease in *Acacia* BNF, and (3) $\delta^{15}\text{N}_{\text{xylem sap}}$ is a more immediate signal of BNF than $\delta^{15}\text{N}_{\text{foliage}}$.

Materials and methods

Study sites

The North Australian Tropical Transect (NATT) is one of 14 transects of the International Geosphere Biosphere project

used to interpret ecosystem function along continental gradients. We sampled plant communities along the ‘highway’ NATT (Kanniah et al. 2011) to the east of the established NATT as the main NATT was impassible due to heavy rains. Sampling was conducted primarily in March–April 2003 (wet season), with additional plant tissue sampled in August 2003 (usually dry season, but following a brief period of rain). The NATT coincides with a rainfall gradient and samples were taken along the transect at four sites: *Eucalyptus*-dominated Darwin (Berrimah, 12°26′02″S, 130°55′22″E, mean annual precipitation 1,600 mm, Australian Government Bureau of Meteorology 2012) and Katherine (near Katherine Research Station grazing enclosure experiment, 14°28′23″S, 132°18′11″E, 1,300 mm), Newcastle Waters (near repeater station, 17°22′39″S, 133°24′35″E, 600 mm, with two sub-sites dominated by either *Acacia* or *Eucalyptus*) and *Acacia*-dominated mulga woodland near Alice Springs (Kunoth Paddock, 23°31′01″S, 133°38′57″E, 250 mm, Supplementary Fig. 1).

Vegetation along the transect grades from monsoonal tall-grass tropical savanna woodlands to semi-arid shrublands and low-open woodlands (Williams et al. 1996) and represents the major variation in biophysical environments of north-western Australian savannas. Along the NATT, woody vegetation cover shifts from *Eucalyptus* to *Acacia* domination at $\sim 21.5^\circ$ south (Bowman and Connors 1996). This is hypothesised to be driven largely by minimum temperature (Bowman and Connors 1996) but the ability of *Acacia* to maintain high foliar N concentrations and photosynthesise efficiently under conditions of low water availability (Lajtha and Whitford 1989; Schulze et al. 1998) may contribute to the shift. Solar radiation along the gradient remains constant (Beringer et al. 2011) and light limitation is minimised by a consistently open canopy. Soil type does not change consistently across the gradient and is dominated by sandy to sandy-clay-loam Kandisols interspersed with Vertisols and cracking clays that are considered to be leached and relatively infertile (McKenzie et al. 2004). A comprehensive overview of previous ecological, biogeographical and physiological studies conducted along the NATT is presented in Hutley et al. (2011). Abiotic conditions, vegetation structure/composition and soil parameters for the NATT region are presented in Hutley et al. (2011) and Beringer et al. (2011).

Soil sampling and analysis

At each site, 6–12 soil cores were taken with a hand auger (10 cm diameter) in the intercanopy space to a depth of 60 cm and divided into subsamples of 0–5, 5–10, 10–20, 20–40 and 40–60 cm depths. An additional six samples of the soil microbiotic crust were collected in intergrove space at the Alice Springs site. Soils and crusts were sieved

to 2 mm, dried at 50 °C for 3 days, homogenised in a ball mill (MM-2; Retsch, Haan, Germany) and analysed for %N and %C via dry combustion and infrared detection in a LECO analyser (CNS-2000; LECO, MI, USA). A subset of 0–10 cm depth soils were analysed for $\delta^{15}\text{N}$ via continuous flow isotope ratio mass spectrometry (Tracer Mass, routine precision 0.2 ‰ SD; Europa Scientific, now PDZ Europa, Sandbach, UK).

The $\delta^{15}\text{N}$ of the sample relative to the standard (atmospheric N_2) was expressed as the following:

$$\delta^{15}\text{N} = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000;$$

where R is the isotope ratio ($^{14}\text{N}/^{15}\text{N}$) and R_{standard} is the $^{14}\text{N}/^{15}\text{N}$ ratio for atmospheric N_2 .

Samples were weighed before and after drying to determine moisture content. To determine soluble inorganic N, soils were sieved and extracted by shaking for 1 h in 1:4 w/v 2 M KCl (Sigma-Aldrich, Sydney, Australia) and centrifuged for 10 min. The supernatant was separated and analysed for NH_4^+ by a modified phenol-hypochlorite method (Westburn 1971) and for NO_3^- using a microtitre plate spectrophotometer method (Miranda et al. 2001). All soil data presented are for March–April 2003.

Plant sampling and analysis

Species in three prominent plant families were sampled at four sites along the transect: (1) diazotrophic, arbuscular/ecto-mycorrhizal *Acacia* species (Fabaceae, including *Acacia shirleyi* Maiden, *A. kempeana* F. Muell., *A. aneura* F. Muell. ex Benth., *A. lysiphloia* F. Muell., *A. trachyphloia* Tindale, *A. neurocarpa* A. Cunn. ex Hook, *A. holosericea*, A. Cunn. ex G. Don and *A. auriculiformis* A. Cunn. ex Benth.); (2) arbuscular/ecto-mycorrhizal *Eucalyptus* and *Corymbia* species (Myrtaceae, including *Eucalyptus capricornia* D.J. Carr & S.G.M. Carr, *E. terminalis* F. Muell., *E. chlorophylla* Brooker & Done, *E. patellaris* F. Muell., *E. tetradonta* F. Muell., *E. miniata* Cunn. ex Schauer, *C. foelscheana* F. Muell., *C. greeniana* D.J. Carr & S.G.M. Carr and *C. dichromophloia* F. Muell.); and (3) non-mycorrhizal *Hakea*, *Grevillea* and *Persoonia* species (Proteaceae, including *Hakea lorea* R. Br., *H. arborescens* R. Br., *Grevillea wickhamii* Meisn., *G. pteridifolia* Knight, *G. dimidiata* F. Muell., *G. striata* R. Br. and *Persoonia falcata* R. Br.). At each site, 4–14 individual trees representing 2–4 species from each of the three plant families were sampled for foliage and shoot and root xylem sap. Xylem samples taken from the same individual trees in March–April and August 2003 were pooled for analysis due to low sample volume.

Fully expanded leaves and phyllodes were sampled from mature trees, air-dried in the field, dried at 50 °C for 3 days,

homogenised and analysed for %N and $\delta^{15}\text{N}$ as above. Xylem sap was extracted from canopy branches and roots of approximately 1 cm diameter with a handheld vacuum pump (Pate et al. 1994). Lateral roots were traced from the trunk outward until they narrowed to ~1 cm diameter in the top 0–30 cm, with most roots occurring in the top 10 cm. Each branch or root yielded 200–1,500 μl sap. Samples were immediately transferred into 1.5 ml O-ring tubes and stored in liquid N_2 . Upon return to the laboratory, samples were stored at -80 °C and analysed for amino acids, NH_4^+ and NO_3^- as per Schmidt and Stewart (1998). A subsample of xylem sap was pipetted into tin cups and freeze-dried in portions of 0.1 cm^3 to yield sufficient N for $\delta^{15}\text{N}$ analysis (performed as above). The proportion of NO_3^- in xylem sap was calculated as percentage of the total low molecular weight N compounds.

Search for root nodules during root excavations for xylem sap occurred by tracing large roots from the bole of approximately five *Acacia* individuals per site to a depth of 30–40 cm. Where they occurred, nodules were visually assessed for activity (non-senescence) based on appearance and coloration.

Statistical analysis

Statistical analyses were based on one-way, two-way or three-way analysis of variance with Tukey's adjusted HSD post hoc tests. The main factors tested in the analysis were plant family, sampling site and/or plant tissue. Where ANOVA assumptions were violated with untransformed data, as detected by non-normal residuals or non-homogeneous variances, the response variable was transformed by the Box–Cox procedure or log-transformation to normalise residuals and homogenise variances. All ANOVA tables were computed based on Type-III sums of squares with orthogonal contrasts on the row-basis of the model matrix. Therefore, significant main effects of one factor may be interpreted meaningfully as average across levels of the other factor, even in the presence of significant interactions (Fox 1997). Interactions are described in the “Results” only where they are statistically significant. Statistics were computed in R 2.9.1 (R Development Core Team 2009), with contributed packages ‘car’ 1.2–16 (Fox et al. 2009) and ‘MASS’ (Venables and Ripley 2002).

Results

Total N, soluble N and $\delta^{15}\text{N}$ of soil

Soil %N varied by site and depth (two-way ANOVA, both $P < 0.001$, $n = 138$, $F = 23.33$ and 165.98). The 0–5 cm soil depth in *Eucalyptus*-dominated savanna showed a

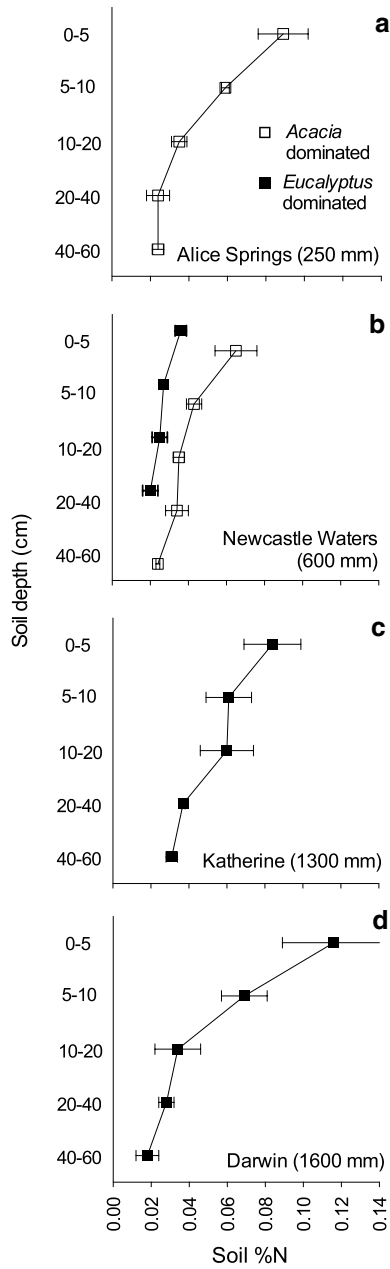


Fig. 1 Soil N content (%N) at **a** Alice Springs, **b** Newcastle Waters, **c** Katherine and **d** Darwin under vegetation dominated by *Acacia* (open symbols) or *Eucalyptus* (filled symbols). Average \pm 1SD of 3–5 soil samples from 3–5 sub-sites are shown. Mean annual precipitation is indicated for each site

significant steep decline in %N along the precipitation gradient, decreasing from 0.12 % (1,600 mm rainfall) to 0.04 % (600 mm; Fig. 1). At the site where *Eucalyptus* and *Acacia*-dominated savanna occurred side by side within the same precipitation range (600 mm), *Acacia*-dominated savanna had significantly (on average 50 %) greater topsoil N (Tukey’s post hoc test, $P < 0.001$; Fig. 1). Soil N content in *Acacia*-dominated savanna increased significantly

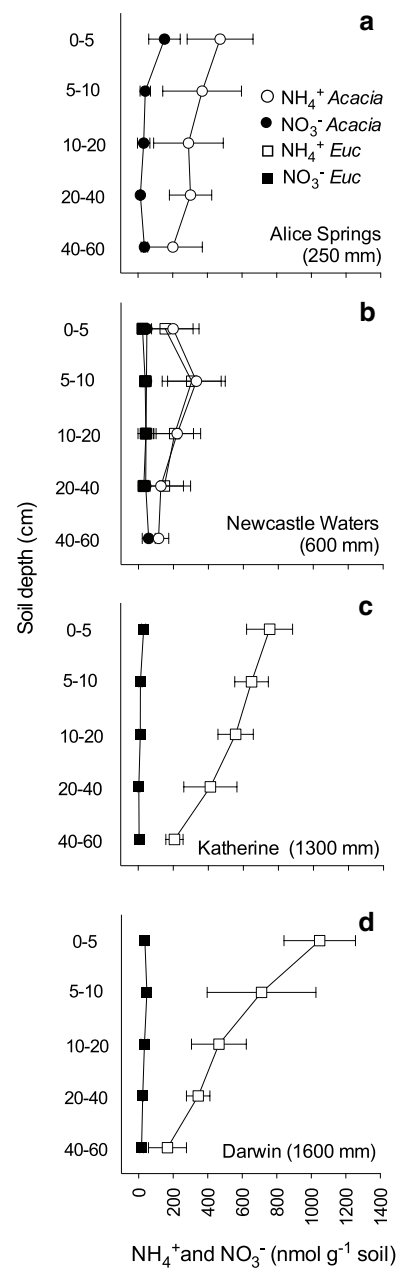


Fig. 2 KCl-extractable soil NH_4^+ (open symbols) and NO_3^- (filled symbols), nmol N g^{-1} soil) at **a** Alice Springs, **b** Newcastle Waters, **c** Katherine and **d** Darwin under vegetation dominated by *Acacia* (circles) or *Eucalyptus* (square symbols). Data represent average \pm 1SD of 5 samples per depth, per site. Mean annual rainfall is indicated for each site

to 0.09 % at the most arid site (250 mm, Tukey’s post hoc test, $P < 0.001$). All sites had consistently low N at depths >20 cm (0.02–0.04 % N).

KCl-extractable soil NH_4^+ followed a similar pattern to %N, differing significantly across sites and depths (two-way ANOVA, both $P < 0.001$, $n = 204$, $F = 37.32$ and 35.31, respectively). NH_4^+ decreased with declining

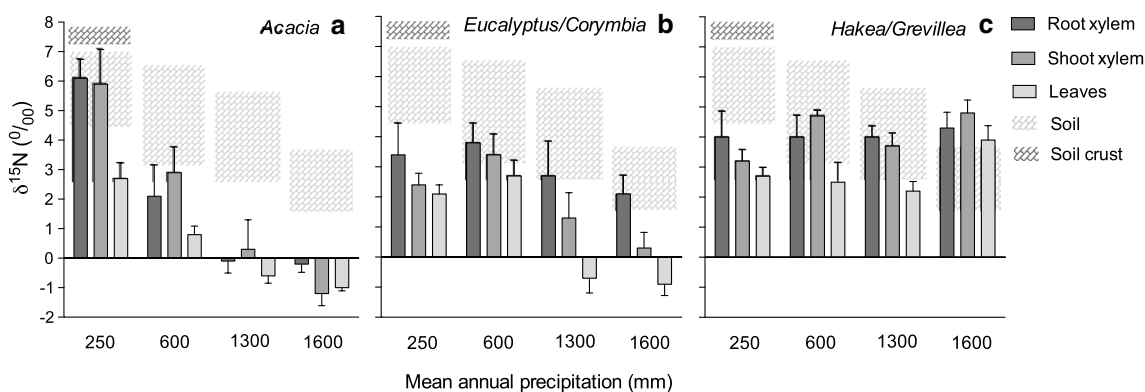


Fig. 3 $\delta^{15}\text{N}$ of root xylem sap, shoot xylem sap and foliage in three woody plant taxa at sites with differing mean annual rainfall (**a** *Acacia*, **b** *Eucalyptus/Corymbia*, **c** *Hakea/Grevillea*). Grey rectangles show the average \pm 1SD of soil $\delta^{15}\text{N}$ in the top 10 cm of the profile

under all plant taxa. Dark rectangle at 250 mm site indicates $\delta^{15}\text{N}$ of microbiotic crust. Data represent average \pm 1SE for 4–14 samples from 2–4 species for each taxa and site

rainfall in *Eucalyptus*-dominated savanna from a maximum of 1,050 $\text{nmol NH}_4^+ \text{g}^{-1}$ soil at 1,600 mm (0–5 cm depth), to 150 $\text{nmol NH}_4^+ \text{g}^{-1}$ soil at 600 mm (Fig. 2). In *Acacia*-dominated sites, concentration increased with declining rainfall, from 160 $\text{nmol NH}_4^+ \text{g}^{-1}$ at the 600 mm site to 474 $\text{nmol NH}_4^+ \text{g}^{-1}$ at 250 mm (0–5 cm depth, $P < 0.001$, Tukey's post hoc test).

As NH_4^+ concentrations decreased, modest increases in NO_3^- concentrations shifted the relative proportions of inorganic N forms in soils across the gradient (Fig. 2). NH_4^+ dominated the soil inorganic N pool at all sites, though the proportion of NH_4^+ across the 0–60 cm soil depth range decreased from 91–97 and 96–99 % (at 1,600 and 1,300 mm rainfall) to 83–89 % at 600 mm in *Eucalyptus*-dominated savanna. In *Acacia*-dominated savanna, NH_4^+ was comparatively less dominant, comprising 66–87 % N at 600 mm rainfall and 76–95 % at 250 mm (0–60 cm values). Topsoil NO_3^- concentrations were higher in *Acacia*-dominated savanna at 250 mm rainfall (153 $\text{nmol NO}_3^- \text{g}^{-1}$, 0–5 cm depth) than at all other sites (Fig. 2; $P < 0.001$, Tukey's post hoc test).

$\delta^{15}\text{N}_{\text{soil}}$ (0–10 cm) also varied by site (one-way ANOVA, $P < 0.001$, $n = 40$, $F = 7.85$) increasing from an average of 2.8 ‰ (range 1.7–3.7 ‰) at 1,600 mm rainfall to 5.6 ‰ (range 4.4–6.9 ‰) at 250 mm (Fig. 3). Obvious microbiotic soil crust was present only at the driest site (250 mm), and $\delta^{15}\text{N}_{\text{crust}}$ (7.8 ‰) was significantly enriched compared to the topsoil (Student's t test, $P < 0.01$).

Nodule morphology, nitrogen content and $\delta^{15}\text{N}$ of plant tissues

No *Acacia* root nodules were observed at the two drier sites (250 and 600 mm). At the two wetter sites (1,600 and 1,300 mm), active root nodules were identified at both sampling time points.

Of the three plant taxa, *Acacia* showed the greatest range and variation in tissue $\delta^{15}\text{N}$ (particularly xylem sap), exhibiting more enriched values at the drier sites. This contrasts with *Hakea/Grevillea*, which exhibited relatively stable tissue isotope values independent of shifts in rainfall and soil $\delta^{15}\text{N}$ values. A three-way ANOVA of all plant $\delta^{15}\text{N}$ values showed a significant main effect of site, plant family and plant tissue (all $P < 0.001$, $n = 310$, $F = 35.12$, 49.64 and 43.01, respectively), as well as significant interactions between site and family ($P < 0.001$, $F = 22.15$). Though $\delta^{15}\text{N}$ of tissues within plant taxa generally showed comparable patterns in response to rainfall, the magnitude of the isotopic offset (net fractionation) between tissues was not constant within or between taxa (Fig. 3). This variable relationship was most pronounced between $\delta^{15}\text{N}_{\text{root xylem sap}}$ and $\delta^{15}\text{N}_{\text{foliage}}$. In *Acacia*, offset between $\delta^{15}\text{N}_{\text{root xylem sap}}$ and $\delta^{15}\text{N}_{\text{foliage}}$ was lower at the low rainfall sites (averaging 0.8 and 0.5 ‰ at the 250 and 600 mm sites, respectively), and increased to 1.3 and 3.4 ‰ at the wetter sites (1,300 and 1,600 mm, respectively; Fig. 3a). *Eucalyptus/Corymbia* exhibited a generally opposing pattern, with the offset decreasing from 3.0 ‰ at the 250 mm site and 3.4 ‰ at the 600 mm site to 1.1 and 1.3 ‰ at the 1,300 and 1,600 mm sites, respectively (Fig. 3b). As a result, $\delta^{15}\text{N}_{\text{foliage}}$ differed significantly between putative N-fixing *Acacia* and non-fixing *Eucalyptus/Corymbia* only at the 600 mm site ($P < 0.01$, Tukey's post hoc test following two-way ANOVA of site \times family, both $P < 0.001$, $n = 128$, $F = 32.15$ and 66.40), though $\delta^{15}\text{N}_{\text{root xylem sap}}$ was distinct in all cases (all $P < 0.01$, analysis as previously, both $P < 0.001$, $n = 72$, $F = 17.83$ and 20.43; Fig. 3a, b). Non-mycorrhizal *Hakea/Grevillea* species showed the least variation with respect to rainfall, with mean $\delta^{15}\text{N}_{\text{root xylem sap}} - \delta^{15}\text{N}_{\text{foliage}}$ offset values ranging by a maximum of 1.7 ‰, and the two tissues significantly distinct only at the 1,600 mm site ($P < 0.05$, Tukey's post hoc test following two-way ANOVA,

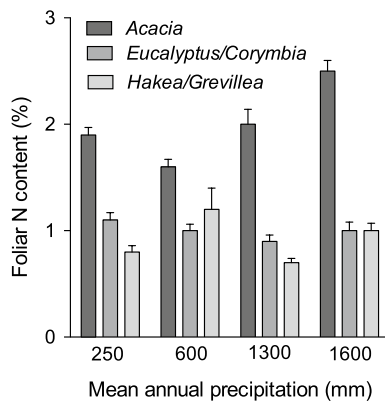


Fig. 4 Leaf N content (%N of dry mass) of three woody plant taxa at sites with differing mean annual rainfall. Data represent average \pm 1SE for 4–14 individual trees per taxa (representing 2–4 species) per site

$n = 106$; Fig. 3c). No significant isotopic fractionation occurred between root and shoot xylem sap in any of the studied species and in general it was not possible to predict which would be enriched/depleted with respect to the other. Samples were pooled between March–April and August and these values may integrate some seasonal variation.

For *Acacia*, there was a significant difference between $\delta^{15}\text{N}_{\text{root xylem sap}}$ and $\delta^{15}\text{N}_{\text{soil}}$ (0–10 cm) at the two wettest sites (1,300 and 1,600 mm; $P < 0.001$ and < 0.01 , Tukey's post hoc test following two-way ANOVA, $n = 145$), where $\delta^{15}\text{N}_{\text{root xylem sap}}$ fell within the range reported for symbiotically fixed N (Robinson 2001, -0.1 ± 0.4 and -0.2 ± 0.3 ‰ at 1,300 and 1,600 mm respectively, Fig. 3a). At the two drier sites, and in all cases for *Eucalyptus/Corymbia* and *Hakea/Grevillea*, soil and root xylem sap $\delta^{15}\text{N}$ did not differ significantly from each other.

Overall, foliar N concentration differed between plant families and sites (two-way ANOVA, both $P < 0.001$, $n = 168$, $P < 0.001$, $F = 42.02 = 9.55$), with a significant interaction between these factors ($P < 0.001$, $F = 9.41$; Fig. 4). The foliar N content of *Acacia* varied between sites (one-way ANOVA, $P < 0.001$, $n = 32$, $F = 113.7$), decreasing from 2.5 to 1.9 % (1,600 and 250 mm sites, respectively). At the 1,600, 1,300 and 250 mm rainfall sites, *Acacia* phyllodes had ≈ 2 -fold higher foliar N concentration than co-occurring *Eucalyptus/Corymbia* or *Hakea/Grevillea* (Fig. 4). *Acacia* foliar N concentrations (but not those of other families) showed a significant relationship with topsoil %N (0–10 cm, linear regression $P < 0.05$, $r^2 = 0.95$).

Nitrate in xylem sap

A two-way ANOVA of shoot xylem sap NO_3^- concentrations showed significant effects of plant family and

site along the rainfall gradient (both $P < 0.001$, $n = 83$, $F = 15.64$, $= 10.27$), and a significant interaction ($P < 0.001$, $F = 5.83$). The percentage of NO_3^- in xylem sap (out of amino acids plus NO_3^-) was significantly elevated in *Acacia* shoots at the lowest rainfall site compared with both other families and with *Acacia* itself at other sites (Tukey's post hoc test, $P < 0.001$; Fig. 5). Though not statistically significant, root xylem % NO_3^- was also elevated in *Eucalyptus/Corymbia* and *Hakea/Grevillea* at the lowest rainfall site.

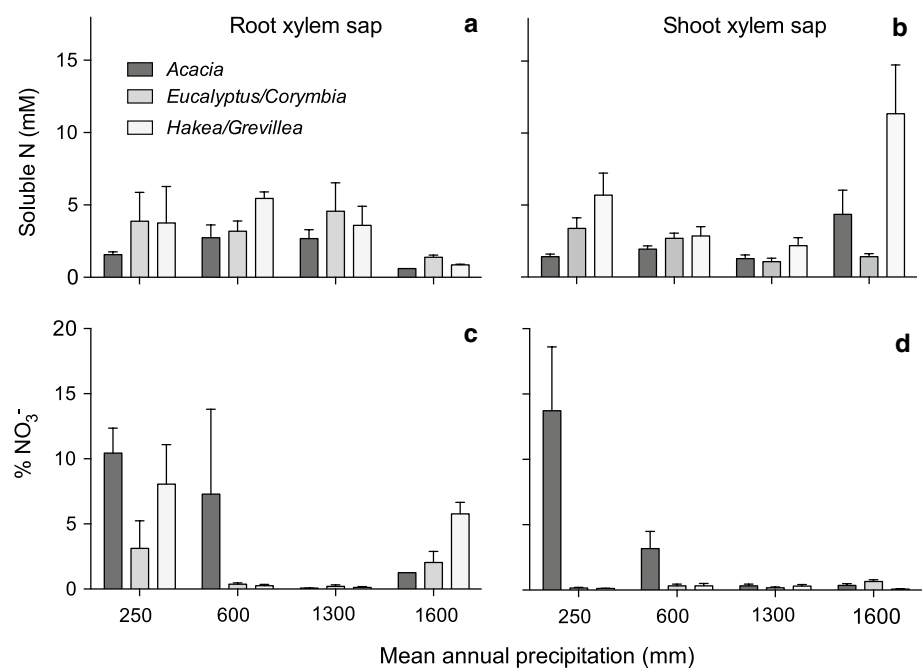
Discussion

Soil N availability and N cycling

Higher soil $\delta^{15}\text{N}$ and proportion of nitrate versus ammonium in drier soils along the North Australian Tropical Transect support the hypothesis that declining rainfall drives increasingly open soil N cycling, validating predictions and mirroring a trend seen across diverse ecosystems (Aranibar et al. 2004; Bai and Houlton 2009; Craine et al. 2009; Handley et al. 1999; McCulley et al. 2009). This openness is likely driven in part by reduced N demand by vegetation, reflecting declining GPP with aridity (Hutley et al. 2011; Kanniah et al. 2011). Previous research has suggested that increasing $\delta^{15}\text{N}_{\text{soil}}$ and $\delta^{15}\text{N}_{\text{foliage}}$ along the NATT transect is caused by increasing grazing pressure (Schulze et al. 1998) or fire frequency (Cook 2001). Alternatively, we propose that this relationship is climate-mediated (Austin and Sala 1999) and driven by relatively greater gaseous losses of isotopically light N with increasing aridity. The drier end of the transect is characterised both by episodic pulsed rain events that are known to promote highly fractionating denitrification losses (Austin et al. 2004) and by higher soil nitrate levels (at the 250 mm site) that may provide a substrate pool for rapid denitrification upon soil wetting. Where gas fluxes occur, the enrichment effect will be relatively greater for a smaller soil N pool at the more arid sites. Additionally, decreasing BNF by *Acacia* and/or microbiotic soil crust along the gradient could reduce inputs of isotopically light N, reinforcing the decline in soil $\delta^{15}\text{N}$ with aridity. In climatically similar sites in Australia, BNF by microbiotic crust is suggested by $\delta^{15}\text{N}$ values which are depleted relative to topsoil (Schmidt et al. 2010). At the driest site of this transect, the isotopic enrichment of crust compared with topsoil implies that that active BNF is not occurring or alternatively that losses of isotopically light N are sufficient to mask the isotope signal of BNF.

At 600 mm annual precipitation, *Eucalyptus*- and *Acacia*-dominated vegetation exists side by side. *Acacia*-dominated savanna is associated with significantly greater total topsoil N than *Eucalyptus*-dominated savanna, and topsoil

Fig. 5 Total soluble N (amino acids, ammonium and nitrate) concentration and % nitrate in root (a, c) and shoot (b, d) xylem sap of three woody plant taxa at sites with differing mean annual rainfall. Data represent average + 1SE for 3–12 samples per species (representing 2–4 species) per site per tissue



N increases further at the *Acacia*-dominated driest site. This pattern cannot be explained by increasing gaseous N loss and reduced BNF inputs. Instead, Pate et al. (1998) argue that the higher N status of mulga woodlands is a relic of times when higher rates of BNF resulted in accrual of N prior to the disturbance of microbiotic soil crusts by widespread grazing of domestic animals (Evans and Ehleringer 1993). Coupled with decreasing GPP and biomass along the transect (Hutley et al. 2011; Kanniah et al. 2011), our observations support the conclusion that N may be more available but is relatively more limiting in wet savanna than semi-arid woodland.

$\delta^{15}\text{N}$ of woody taxa

Comparisons of $\delta^{15}\text{N}$ in root and shoot xylem sap and foliage revealed significant and variable patterns of net fractionation between N pools, both between and within taxa, which hinder interpretation of foliar values as a reflection of plant N nutrition. Though the direction of fractionation remained constant, separation between $\delta^{15}\text{N}_{\text{root xylem sap}}$ and $\delta^{15}\text{N}_{\text{foliage}}$ was much greater in *Eucalyptus/Corymbia* at the two wetter than the two drier sites, while *Acacia* displayed the opposite pattern. As a result, N-fixing *Acacia* and non-fixing *Eucalyptus/Corymbia* have depleted, overlapping foliar $\delta^{15}\text{N}$ values at the wetter sites. This scenario would preclude traditional application of the foliar $\delta^{15}\text{N}$ method as a means of assessing BNF (Boddey et al. 2000; Shearer and Kohl 1986). However, for $\delta^{15}\text{N}_{\text{root xylem sap}}$ (a pool that precedes fractionation of N in foliage), *Acacia* displays a

pattern consistent with increasing BNF with precipitation while *Eucalyptus/Corymbia* remains stable and enriched over time and is consistent with lower flexibility in N nutrition.

Several mechanisms may contribute to fractionation of N between xylem and foliar tissue, including distribution of nitrate reduction between roots and shoots (Andrews 1986; Comstock 2001; Tcherkez and Hodges 2008; Werner and Schmidt 2002), leaf N turnover and export (Gauthier et al. 2012; Tcherkez 2011), rate of photorespiration (Tcherkez and Hodges 2008) and age (Kolb and Evans 2002). There is also a temporal disconnect between xylem N which represents recently acquired N with a turnover time of hours to days (Hayashi et al. 1997) and leaves which integrate N nutrition over months to years when N availability may have been different to the present (Evans 2001a, b; Gebauer and Schulze 1991). However, this disconnect alone is unlikely to explain the large differences in fractionation observed here as we have also observed sustained differences between foliar and xylem $\delta^{15}\text{N}$ values over time independent of N availability in other ecosystems (Soper et al. 2014). Regardless of the mechanism, physiological differences between taxa and their interaction with environmental conditions are sufficient to cause significant variation in foliar fractionation patterns. In this context, $\delta^{15}\text{N}_{\text{root xylem sap}}$ may provide useful information on patterns of N acquisition by preceding major fractionation steps, though interpretation should be not considered fully quantitative unless fractionation occurring during uptake from the soil and prior to xylem loading can be accounted for.

Biological N fixation in *Acacia*

Several observations support the hypothesis that BNF in *Acacia* species declines with aridity, potentially driven by both water availability and plant and soil N status. At the two wetter sites, observations of active root nodules were accompanied by $\delta^{15}\text{N}_{\text{root xylem sap}}$ values of 0 to -1‰ (fixed N compounds transported in root xylem sap retain $\delta^{15}\text{N}$ values close to zero; Bergersen et al. 1988). At the two drier sites, *Acacia* root nodules were absent and $\delta^{15}\text{N}_{\text{root xylem sap}}$ fell within the range for non-fixing species. The increased proportion of nitrate observed in *Acacia* root and shoot xylem sap (which must be soil-derived as plants do not produce nitrate in vivo; Smirnov and Stewart 1985) at the two driest sites has two implications. Firstly, nitrate accumulation by *Acacia* in semi-arid mulga has been proposed as a mechanism of osmotic regulation that facilitates adjustment to low soil water potentials, an environmental condition known to reduce N fixation in the *Rhizobium*-legume symbiosis (Erskine et al. 1996; Hartwig 1998; Serraj et al. 1999; Zahran 1999). Secondly, greater soil concentrations of inorganic N, particularly nitrate, directly suppress nodulation in many legume species (Hartwig 1998; Hedin et al. 2009). In lowland tropical forests, Barron et al. (2011) observed a significant decline ($\sim 50\%$) in nodule abundance of woody legumes (*Inga* sp.) at soil nitrate concentrations equivalent to those measured at the driest site in our study (2.1 mg NO_3^- per kg soil). Increased nitrate uptake by *Acacia* at the two drier sites can likely be excluded as a direct explanation for the trend towards more enriched *Acacia* tissue $\delta^{15}\text{N}$ values with aridity. Though predicting $\delta^{15}\text{N}$ -nitrate is not straightforward (the pool integrates depleting fractionation occurring during nitrification as well as enrichment from soil gaseous losses; Högberg 1997), other studies of northern Australian ecosystems have observed that soil $\delta^{15}\text{N}$ -nitrate is depleted with respect to $\delta^{15}\text{N}$ -ammonium (Pate et al. 1998; Schmidt 1996). Thus, increased nitrate uptake would be expected to counter rather than reinforce the pattern we observed. Additionally, the proportion of nitrate in xylem sap showed no correlation with $\delta^{15}\text{N}$ values in *HakealGrevillea*. Overall, we conclude that decreasing BNF by *Acacia* provides the most parsimonious explanation for our suite of observations and supports previous interpretations (Schulze et al. 1998). Taken together, we propose that a shift from N to water limitation with declining rainfall decreases *Acacia* BNF along the gradient, and that flexibility in N acquisition strategy in response to availability and environmental conditions enables *Acacia* to maintain high foliar N concentrations and support a N-demanding lifestyle (Sprent 1994).

N nutrition of *Eucalyptus/Corymbia* and *HakealGrevillea*

Non-mycorrhizal *HakealGrevillea* showed surprising consistency in $\delta^{15}\text{N}_{\text{root xylem sap}}$ with declining rainfall, unrelated to increasing bulk $\delta^{15}\text{N}_{\text{soil}}$. Non-mycorrhizal plants (including Proteaceae) typically experience less isotopic fractionation upon N uptake than mycorrhizal species (Craine et al. 2009; Evans 2001a, b; Hobbie and Högberg 2012) and so plant tissue should theoretically track the soil pool most closely. Although $\delta^{15}\text{N}_{\text{soil}}$ is not necessarily reflective of the isotopic signature of plant available inorganic N, it is influenced by the relative long-term rates of mineralisation, nitrification and gaseous loss (in turn linked to rainfall and soil water availability; Boddey et al. 2000; Evans 2007; Högberg 1997). Cluster roots in *HakealGrevillea* facilitate access to complex organic N (Paungfoo-Lonhienne et al. 2008) and although the relationship between the isotopic composition of bulk soil and various organic N fractions is not well described, we found no evidence of changing N source use for *HakealGrevillea* along the gradient.

If we assume that taxa within a site access soil N of comparable isotopic composition, the more depleted $\delta^{15}\text{N}_{\text{root xylem sap}}$ of mycorrhizal *Eucalyptus/Corymbia* compared to non-mycorrhizal *HakealGrevillea* at the wetter sites is consistent with fractionation of $\delta^{15}\text{N}$ by mycorrhizae during fungal-plant transfer (Hobbie and Högberg 2012; Spriggs et al. 2003). As rainfall decreased, we observed a minor ($< 2\text{‰}$, statistically non-significant) increase in the average $\delta^{15}\text{N}_{\text{root xylem sap}}$ in *Eucalyptus/Corymbia*. The degree of mycorrhizal colonisation of roots (and thus expected fractionation) may be greater at wet sites and decline with aridity, although there is conflicting evidence on the expected relationship between degree of VA-mycorrhizal colonisation and rainfall (Hawkes et al. 2011; Yang et al. 2011). If the increase in *Eucalyptus/Corymbia* $\delta^{15}\text{N}$ were driven solely by a systematic enrichment of the inorganic N pool (mirroring the enrichment of bulk soil $\delta^{15}\text{N}$), we might expect *HakealGrevillea* to show a comparable increase in $\delta^{15}\text{N}_{\text{root xylem sap}}$. However, this did not occur.

Conclusions

Our study provides evidence that declining rainfall along a savanna to woodland gradient in northern Australia correlates with shifting patterns of N availability, leading to a more open N cycle as the plant community transitions from N limitation to water limitation. This transition correlates with an apparent decrease in BNF by dominant woody genus *Acacia* that is supported by measures of tissue $\delta^{15}\text{N}$, nitrate accumulation in xylem sap and nodule occurrence. Our observations suggest minor changes in the nutritional strategies of other dominant taxa *Eucalyptus/Corymbia* and *HakealGrevillea*

despite significant changes in soil N content and availability and a decline in annual precipitation of over 80 %. On an ecosystem scale, the capacity for BNF does not explain *Acacia* dominance in drier areas although flexibility in N acquisition strategies may enable *Acacia* species to compete more successfully than less adaptable woody taxa in drier areas. Increase in topsoil N associated with *Acacia* dominance at the driest sites may reflect historical N inputs and a lack of N demand driven by lower productivity and water limitation, rather than active BNF. Pronounced differences in internal N isotope fractionation within and between taxa demonstrate that $\delta^{15}\text{N}_{\text{root xylem sap}}$ is a more direct measure of plant N nutrition than commonly applied $\delta^{15}\text{N}_{\text{foliage}}$ and should be investigated further. Next steps require more in-depth quantification of other fractionation events affecting the composition of this pool including uptake from the soil and assimilation in roots.

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