

Short communication

Nitrogen fixation by the savanna tree *Philenoptera violacea* (Klotzsch) Schrire (Apple leaf) of different ages in a semi-arid riparian landscape

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Abstract

The acquisition of nitrogen for growth and maintenance is essential for plants, and having multiple strategies for that acquisition is especially important for those colonizing nutrient poor substrates. *Philenoptera violacea* (Apple leaf) is a prominent tree in nutrient poor savanna and alluvial soils near rivers in southern Africa, where nutrient availabilities are highly variable in space and time. We investigated nitrogen fixation in *P. violacea* within riparian corridors flanking the Sabie River in Kruger National Park (KNP) in the Lowveld in northeastern South Africa using the natural ^{15}N abundance technique. Results indicated that *P. violacea* fixes atmospheric nitrogen and this varies with life history stage. We found that foliar $\delta^{15}\text{N}$ levels were significantly lower in all life stage classes of *P. violacea* compared with the reference plant *D. mespiliformis* growing in open riparian forest. In addition $\delta^{15}\text{N}$ values were significantly different within the different life stages of *P. violacea* with the leaves of saplings and juvenile plants having significantly lower $\delta^{15}\text{N}$ levels than mature plants. While $\delta^{15}\text{N}$ values increased with age, foliar nitrogen concentration values declined, with leaves from sapling *P. violacea* having significantly higher total nitrogen than adults and juveniles, which were in turn significantly higher than juvenile *D. mespiliformis*. However, foliar $\delta^{15}\text{N}$ levels in seedlings of *P. violacea* growing in a high nutrient environment in flood debris piles did not differ from levels recorded in seedlings of the reference tree. This study confirms that *P. violacea* is able to fix nitrogen, but it is dependant on soil conditions and the life stage of the trees.

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1. Introduction

Nitrogen is a major requirement for optimal plant growth, and a major theme in ecological research. However, the role of symbiotic nitrogen fixation of woody and herbaceous legumes in the biogeochemical cycles of semi-arid African savannas is a matter of more debate. A large fraction of the basal area of the woody component of savanna in semi-arid South Africa consists of deciduous and semi-deciduous legumes (Scholes and Walker, 1993; Scholes et al., 2003). A dichotomy in the ability to fix atmospheric nitrogen within the legumes has

become an accepted paradigm, with woody members of the Mimosoideae and Papilionoideae able to fix nitrogen and members of the Caesalpinioideae unable to do so (Aranibar et al., 2003). However recent work has suggested that the situation is more complex with some species of the Caesalpinioideae appearing to fix nitrogen while some members of Mimosoideae and Papilionoideae do not (Diabate et al., 2005). Further, root excavations of individuals from key woody leguminous species in South African savannas, including members of the Mimosoideae have failed to locate nodules in any great number, leading to speculation that these plants do not fix nitrogen at all (Scholes et al., 2003). However not all species that are known to produce root nodules will do so under all circumstances (Diabate et al., 2005). This can depend on soil conditions and the presence of suitable endophytic bacteria (Sprent, 2005). Compounding the environmental complexity is

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the differential availability of soil nutrients in the highly heterogeneous and patchy savanna ecosystems in southern Africa (Scholes et al., 2003).

Philenoptera violacea (Klotzsch) Schrire (Apple leaf), a semi-deciduous member of the Fabaceae family (subfamily Papilionoideae) is a prominent tree in nutrient poor savanna and alluvial soils near rivers in southern Africa (Van Wyk and Malan, 1998; Fig. 1a). A previous study in the Lowveld savanna has shown an inability to fix nitrogen for the few individuals of mature *P. violacea* sampled (Aranibar et al., 2003). However relatively high foliar nitrogen concentrations in excess of 3% have been measured for *P. violacea* (du Toit, 2003; S.M. Jacobs unpublished data). These levels of foliar nitrogen, which were well in excess of levels in neighbouring non-leguminous plants indicates that this species may have the ability to fix atmospheric nitrogen. However it has also been suggested that nitrogen fixers in general have a nitrogen demanding lifestyle in that they require high levels of nitrogen whether or not nitrogen is fixed (Vitousek et al., 2002). Clarification of the ability of *P. violacea* to fix nitrogen will aid in understanding spatial patterns in fertility and the role of leguminous woody trees in nitrogen cycling, especially in riparian corridors (S.M. Jacobs and R.J. Naiman, unpublished data).



Fig. 1. (a) Mature *Philenoptera violacea* in the riparian environment of the Sabie River. (b) *P. violacea* seedlings growing in a woody debris pile at Site 2.

Stable isotopes analysis of foliar material using ^{15}N provides a qualitative measure of nitrogen fixation as it indicates the ability of plants to fix atmospheric nitrogen (Nadelhoffer and Fry, 1994; Högberg, 1997; Dawson et al., 2002). As the fractionation factor for nitrogen fixation in the root nodule is low, $\delta^{15}\text{N}$ values close to zero is usually an indication of fixation of atmospheric nitrogen (used as standard in measuring ^{15}N) in legumes (Nadelhoffer and Fry, 1994). This index is usually consistent with the presence of nitrogen fixing activity of root nodules containing the nitrogen fixing bacteria. In most ecosystems, plants deriving nitrogen from sources other than atmospheric nitrogen are characterized by values elevated above those of nitrogen fixing species, reflecting the signal associated with nitrogen from the soil pool (Dawson et al., 2002).

Our goal was to use the natural ^{15}N abundance technique to determine the nitrogen fixing ability of *P. violacea* growing in the riparian corridor of granitic savanna in the South African Lowveld. We used nitrogen isotopes of collected foliar material from *P. violacea* trees and foliar material from a reference non-nitrogen-fixing tree, jackalberry (*Diospiros mespiliformis* Hochst.) to gain an understanding of the range in nitrogen isotopes amongst seedlings, saplings, juvenile and mature specimens. We asked the questions: (i) Is *P. violacea* capable of fixing atmospheric nitrogen in the riparian habitat of the Sabie River? (ii) Does nitrogen fixing vary with life stage of the plants?

2. Methods

2.1. Study sites

We worked within riparian corridors flanking the Sabie River within KNP in the Lowveld savanna in northeastern South Africa. The two study sites are situated in the riparian zone on the toeslopes of granitic catenas. Soils derived from granites are generally low in nutrients, though toeslopes typically contain higher nutrients than elsewhere along the catena (Venter, 1990). Our first site (Site 1; 7234882.3N, 376240.9E) was located in the open riparian forest within a large animal enclosure from where all large herbivores (>5 kg) had been recently removed. Sampling was carried out in July 2005, 3 years after large herbivore exclusion commenced (O'Keefe and Alard, 2003). Ten sampling points were located along a 700-m reach of the Sabie River and an adjacent tributary. At each sampling point plants of three different ages of *P. violacea* were sampled, saplings (<0.9 m high), juvenile trees (between 1.5 to 7 m) and mature trees (7 to 10 m). One size class (juvenile, 4 to 10 m) of the reference tree *D. mespiliformis* trees was also sampled. At each sampling point the nearest plant for each category was sampled (i.e. four trees were sampled at each sampling point, including sapling, juvenile and adult of *P. violacea* and juvenile of *D. mespiliformis*). As it was not possible to determine the age of the individual leaves, we sampled a large number of leaves (50–100) from three branches in the top third of the northern side of each tree.

A second site (Site 2; 7239028.1N, 372754.3E) was located within a large woody debris pile formed by debris from a large flood that occurred in 2000 (Heritage et al., 2001). Nutrients are generally higher in riparian debris piles than the neighbouring soil matrix (Pettit and Naiman, 2005). Debris piles thus act as patches for enhanced environmental resources within savanna riparian zones. We collected recently germinated (<1 year) *P. violacea* (Fig. 1b) and *D. mespiliformis* seedlings associated with the debris pile to determine if *P. violacea* seedlings were fixing nitrogen in a high-nitrogen environment.

All samples were stored in paper bags and dried in a warm room (38° to 43 °C) equipped with a dehumidifier and a heater. After 7 days, all samples were transferred to a convection oven and dried further at 45 °C for 1 day. Samples were then ground in a rotary mill. Powdered leaf samples were analyzed for %N and $\delta^{15}\text{N}$ by combustion in an automated Elemental Analyzer (Carlo-Erba), and N_2 gas resulting from this process introduced to a Mass Spectrometer (MAT 252 or DELTA XP) using a continuous flow-through inlet system.

Nitrogen fixation was indicated by the isotope ratio, using the following formula:

$$\delta^{15}\text{N} = 1000 * [(R_{\text{sample}}/R_{\text{atmosphere}}) - 1]$$

where R is the molar ratio $^{15}\text{N}/^{14}\text{N}$ and the units are per mil (‰). A $\delta^{15}\text{N}$ ratio close to zero in the leguminous samples indicates atmospheric nitrogen fixation when compared to more positive ratios in the reference plant, which indicates use of nitrogen from the soil pool (Dawson et al., 2002).

At the first site, soil was sampled from the A horizon, with sample points generally situated within 40 m of each tree sample. Soil samples were sieved through a 2-mm sieve, air-dried and analysed for total nitrogen (%) using a Carlo-Erba CN analyser after combustion. Pettit and Naiman (2005) reported methods and results for soil nitrogen concentrations inside and outside large woody debris piles along the Sabie River.

2.2. Statistical Analysis

The Statview statistical package (Version 5, SAS Institute Inc. 1998) was used for statistical analyses. One way ANOVA was used to test significant differences in foliar $\delta^{15}\text{N}$ and total nitrogen (dependent variables) of material from the open forest for different combined life stage/species group (independent variable ($df=3$, i.e. seedling, juvenile, adult *P. violacea* and juvenile *D. mespiliformis*)). Where the ANOVA gave a significant result Fishers LSD post-hoc test was calculated to separate which groups were significantly different. In addition an unpaired t test was performed to look at differences in foliar $\delta^{15}\text{N}$ and total nitrogen (%) between juvenile *P. violacea* and *D. mespiliformis* and well as seedlings of each species growing in the wood debris pile.

3. Results

Foliar $\delta^{15}\text{N}$ levels were significantly lower in all life stage classes of *P. violacea* compared with the reference plant

D. mespiliformis growing in open riparian forest (Site 1) ($F=12.44$, $p<0.001$; Fig. 2a). Results showed $\delta^{15}\text{N}$ levels for *P. violacea* ranged between 0.4 and 1.8‰ compared with values between 2.9 and 3‰ for *D. mespiliformis*. To eliminate any confounding effect of plant age foliar $\delta^{15}\text{N}$ levels in juvenile *P. violacea* were compared directly with levels in juvenile *D. mespiliformis* which showed a significantly lower level in *P. violacea* leaves ($t=4.80$, $p<0.001$). In addition $\delta^{15}\text{N}$ values were significantly different within the different life stages of *P. violacea* with leaves of saplings and juvenile plants having significantly lower $\delta^{15}\text{N}$ levels than the mature plants (Fig. 2a). This suggests higher levels of atmospheric nitrogen fixing in the younger plants.

Foliar total nitrogen values showed opposite trends to $\delta^{15}\text{N}$ results with leaves from sapling *P. violacea* having significantly higher total nitrogen than adult and juveniles which were in turn significantly higher than the juvenile *D. mespiliformis* ($F=40.42$, $p<0.001$; Fig. 2a). The differences between leaves of all life stages of *P. violacea* and that of the reference tree

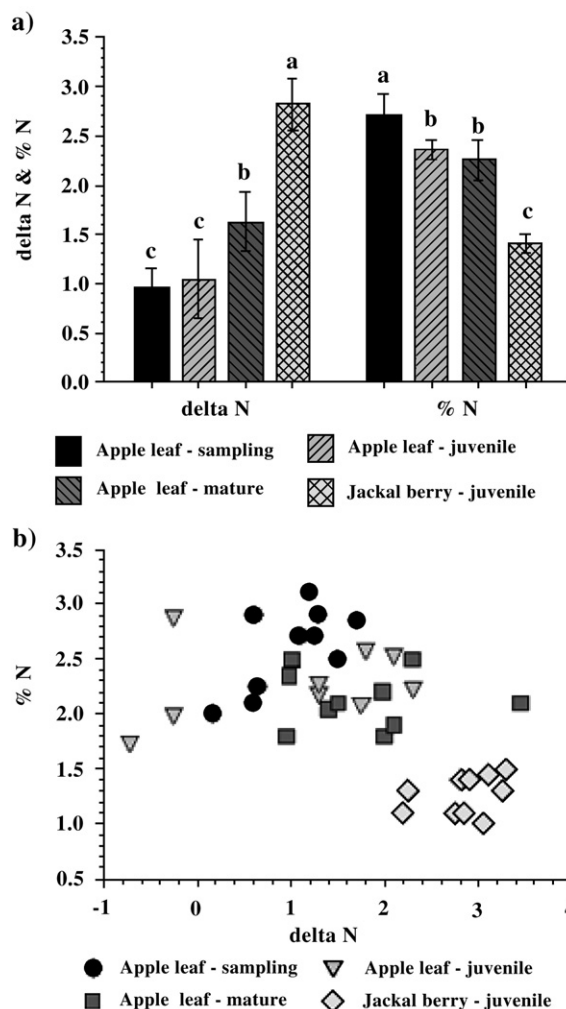


Fig. 2. (a) $\delta^{15}\text{N}$ and total % nitrogen in foliar material from plants growing in open riparian forest. For each variable, columns with the same letter are not significantly different (ANOVA followed by Fishers LSD test). (b) Relationship between the $\delta^{15}\text{N}$ and total % nitrogen for the different life-stage/species groups.

(*D. mespiliiformis*) is clearly shown when $\delta^{15}\text{N}$ values are plotted against total nitrogen percentages (Fig. 2b). This shows the separation with higher % nitrogen values with lower $\delta^{15}\text{N}$ for *P. violacea* compared with the non-N fixing reference tree. Some values for mature *P. violacea* samples are similar to the reference tree (Fig. 2b). This may indicate that older trees may no longer be fixing nitrogen. Indeed a number of the leaves of the mature trees sampled were distinctly less green in colour than samples from younger plants.

Seedlings growing in the large woody debris pile showed mean $\delta^{15}\text{N}$ values of 4.6‰, and did not differ significantly from the reference plant (*D. mespiliiformis*) ($t=0.67$, $p=0.51$; Fig. 2b). This would indicate that they are not fixing atmospheric N. Total foliar nitrogen was however significantly higher in *P. violacea* seedlings compared to the *D. mespiliiformis* seedlings ($t=3.89$, $p<0.001$). Swellings were found on the roots of some excavated seedlings however, we were unable to confirm whether these were *Rhizobium*-containing nodules.

The mean total nitrogen concentration ($n=10$) for surface soils in the open riparian forest at Site 1 was $0.094\pm 0.01\%$. Soil total nitrogen concentrations taken from large woody debris piles ($n=15$) at site two showed significantly higher concentrations ($t=2.2$, $p=0.03$) inside the large woody debris piles ($0.137\pm 0.03\%$) compared with outside ($0.091\pm 0.01\%$), confirming the contention that these patches are hotspots for nitrogen dynamics.

4. Discussion

The evidence presented here suggests that *P. violacea* fixes atmospheric N. Plants deriving nitrogen from sources other than atmospheric nitrogen are characterized by $\delta^{15}\text{N}$ values elevated well in excess of zero (Evans, 2001; Dawson et al., 2002) while nitrogen fixing plants tend to have higher levels of foliar total nitrogen relative to non-N fixers in the same environment (Vitousek et al., 2002). The results also show that atmospheric nitrogen fixing ability may vary with the age of plants and soil nutrient conditions. Both the saplings and juvenile *P. violacea* trees have significantly lower $\delta^{15}\text{N}$ values than mature *P. violacea* trees and the reference trees. Conversely, the sapling and juvenile *P. violacea* plants had higher total nitrogen values than the mature trees and the reference trees. This suggests that the young actively growing plants are more likely to be fixing nitrogen as they have greater requirements for growth. The ability to fix nitrogen where the soil pool is unable to provide the total requirements allows *P. violacea* seedlings to attain high growth rates and to escape from the riparian understorey level and perhaps also browsing from ungulate herbivores (Naiman and Rogers, 1997). In older, slower growing trees, already having escaped from the understorey, high nitrogen levels may not be required. The mature plants of *P. violacea* that were sampled may no longer be growing and may have only minimal requirements for nitrogen for the maintenance of leaf area. These requirements can be accessed from the soil, particularly in the relative nutrient rich riparian soils (Venter, 1990), with nitrogen fixation becoming less important.

P. violacea seedlings growing in the large woody debris pile obtained the majority of foliar nitrogen from the soil. Both *P. violacea* seedlings and the reference plant show

relatively high $\delta^{15}\text{N}$ values, indicating that nitrogen is taken up from the soil pool. *P. violacea* seedlings also seem to be more efficient in taking up nitrogen than *D. mespiliiformis* as foliar nitrogen values were significantly higher (Fig. 3). This is consistent with the observations by Vitousek et al. (2002) that legumes have high demand for nitrogen. It is not surprising that seedlings growing in large woody debris piles did not fix atmospheric nitrogen, as the patches have been shown to be hotspots for nitrogen cycling in riparian zones with total nitrogen, nitrate and ammonium concentrations generally higher in debris piles than in the rest of the riparian corridor (Pettit and Naiman, 2005). A similar trend was observed in stands of red alder (*Alnus rubra* Bong.) where accumulation of nitrate reduced the nodulation capacity of seedlings, here used as an indicator of nitrogen fixing ability of plants growing in the soil substrate (Martin et al., 2003). *P. violacea* seedlings seem to be able to benefit from elevated nitrogen availability in woody debris piles. This may indicate a facultative nitrogen fixing habit for this species, at least at the seedling stage. The process of nitrogen fixation is energetically expensive; so that when nitrogen could be obtained from other sources, this form of uptake

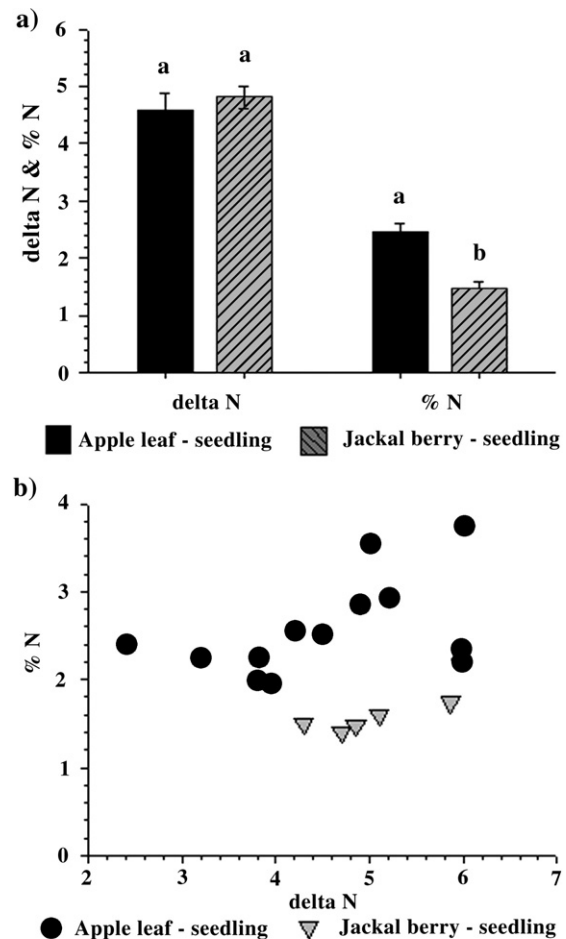


Fig. 3. (a) $\delta^{15}\text{N}$ and total % nitrogen in foliar material from plants growing in large woody debris piles. For each variable columns with the same letter are not significantly different (unpaired t test). (b) Relationship between the $\delta^{15}\text{N}$ and total % nitrogen for the different species.

will be favoured over atmospheric nitrogen fixation (Vitousek et al., 2002). The absence of nitrogen fixation in legumes with root nodules has been reported in other studies (Yoneyama et al., 1984). This may indicate that nitrogen fixation may become an option at different life-history stages of the plant, when nitrogen in the soil pool may be unable to supply the demand. Alternatively the swellings observed in the present study may not be related to plant-*Rhizobium* symbiosis as it is often difficult to ascertain in the field that swellings found on roots are the result of *Rhizobium* infection (Sprent, 2005).

Aranibar et al. (2003) found that *P. violacea* growing in upland areas do not fix nitrogen, although this was from sampling only 1 or 2 plants. Our results show that this species is able to fix nitrogen; however, this seems to be facultative depending on age and soil conditions. When soil nitrogen levels are unable to supply optimal levels, a significant portion of the nitrogen debt can be fixed from the atmosphere. Nitrogen fixation could also be inhibited by a variety of ecological factors, including water and phosphorus availability (Vitousek et al., 2002). Toeslope areas in Lowveld semiarid savannas are known to have elevated soil water levels (February, personal communication)¹ and phosphorus (Venter, 1990; S.M. Jacobs and R.J. Naiman, unpublished data). This may allow elevated rates of nitrogen fixation in lower slope areas. Conversely, nitrogen fixation can be inhibited by elevated soil nitrogen levels. Nitrogen fixing legumes growing in patches with high available soil nitrogen may be able to take up the required nitrogen, thus dispensing with the need to fix nitrogen (Vitousek et al., 2002). Environmental factors and life-history stage of the sample trees may explain differences between what was found in the present study and that of Aranibar et al. (2003).

The variations in the nitrogen fixing levels seen here in *P. violacea* highlight the importance of detailed studies into particular habitats to complement broad-ranging, but relatively superficial studies that can screen a large number of species quickly but may miss detail such as the effects of edaphic factors and age of the plants. The results presented here are also a valuable contribution to the characterization of nitrogen cycling within the riparian zone in Lowveld savanna. However this work on *P. violacea* is by no means definitive and further studies on seasonal differences in nitrogen fixation as well as laboratory based work looking at nodulation and nitrogen fixation of seedlings under different nutrient regimes would be the logical next steps.

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