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Short communication

Estimates of nitrogen fixation by trees on an aridity gradient in Namibia*

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Summary. Nitrogen (N_2) fixation was estimated along an aridity gradient in Namibia from the natural abundance of ^{15}N ($\delta^{15}N$ value) in 11 woody species of the Mimosaceae which were compared with the $\delta^{15}N$ values in 11 woody non-Mimosaceae. Averaging all species and habitats the calculated contribution of N_2 fixation (N_f) to leaf nitrogen (N) concentration of Mimosaceae averaged about 30%, with large variation between and within species. While in *Acacia albida* N_f was only 2%, it was 49% in *Acacia hereroensis* and *Dichrostachys cinerea*, and reached 71% in *Acacia melifera*. In the majority of species N_f was 10–30%. There was a marked variation in background $\delta^{15}N$ values along the aridity gradient, with the highest $\delta^{15}N$ values in the lowland savanna. The difference between $\delta^{15}N$ values of Mimosaceae and non-Mimosaceae, which is assumed to result mainly from N_2 fixation, was also largest in the lowland savanna. Variations in $\delta^{15}N$ of Mimosaceae did not affect N concentrations, but higher $\delta^{15}N$-values of Mimosaeae are associated with lower carbon isotope ratios ($\delta^{13}C$ value). N_2 fixation was associated with reduced intrinsic water use efficiency. The opposite trends were found in non-Mimosaceae, in which N-concentration increased with $\delta^{15}N$, but $\delta^{13}C$ was unaffected. The large variation among species and sites is discussed.

Key words: Nitrogen fixation – Carbon isotope ratio – Nitrogen isotope ratio – *Acacia* – Namibia

Subtropical savannas are characterized by a high proportion of trees of the genus *Acacia* or closely related species of the Mimosaceae family (Walter 1964). The proportion of other tree species may increase towards the more humid as well as towards the more arid regions (Högberg 1986a). Despite the very pronounced occurrence of *Acacia* in this vegetation type, information about the contribution of nitrogen (N_2) fixation towards the nitrogen requirements of these species is scarce. In the Sonoran Desert N_2 fixation may contribute 43–61% to the N content of *Prosopis,* but is was negligible in the genus *Acacia* (Shearer et al. 1983). In Tanzania 44% of the N content of potentially N_2-fixing species originated from N_2 fixation (Högberg 1986b). In both Tanzania and the Sonoran desert the variability of N_2 fixation among species and habitats was very large. It was thought that this results mainly from variation in nodulation and from soil chemical factors such as the availability of phosphorous and from the type of mycorrhiza (Högberg 1986a, b, 1990; Shearer et al. 1983).

In this study we further investigate the variation in $\delta^{15}N$ among Mimosaceae in order to quantify the contribution of N_2 fixation. This study also seeks to investigate the effect of N_2 fixation on water use efficiency. Improved N supply could potentially increase the N content of leaves (Högberg 1986b) and cause stomata to open (Schulze and Hall 1982). So far, the overall effect on water use efficiency is not clear. If stomatal conductance changed in proportion to assimilation, this might not change the intrinsic water use efficiency. Therefore, the carbon isotope ratio ($\delta^{13}C$) was determined as a measure of the intrinsic water use efficiency of leaves (Farquhar et al. 1989). In order to encompass wide variations in nitrogen fixation, trees were studied on an aridity gradient in the Namib desert, extending from the highlands of Namibia to its coast (Giess 1971; Schulze and Schulze 1976; Schulze et al. 1976; Besler 1972).

Material and methods

Fully expanded leaves of trees representing the range of Mimosaceae and non-Mimosaceae at each sampling location were collected along a transect from the highland of Namibia close to Windhoek (240 km from the coast) along the Walvis Bay road to the Namib desert at Sesriem (130 km from the coast) and to the coastal river plain of the Swakop river (50 km from the coast; Table 1). The collection was made in March 1987 at the end of the wet season. At five sampling locations, leaves of at least 10 trees of as many Mimosaceae- and of 10 trees of non-Mimosaceae tree species were

* This paper is prepared in memory of J. Visser, who took part in the collection of species, but died in 1990

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Table 1. Locations of collection of tree species. Plant names according to Merxmüller (1972), rainfall according to Walter (1964)

Location (habitat type)	Distance to coast (km)	Rain fall (mm)	Tree species	
			Mimosaceae	non-Mimosaceae
Dan Feljun Park	240	400	*Acacia erioloba*	*Boscia albitrunca*
(highland savanna)			*A. hebeclada*	*Rhus lancia*
			A. hereroensis	*Ziziphus mucronata*
			A. karroo	
			A. reficiens	
			A. tortilis	
			Dichrostachys cinerea	
Windhoek	200–230	300	*A. hebeclada*	*B. albitrunca*
Walvisbay			*A. hereroensis*	*Rhigozum trichotomum*
(highland savanna)			*A. mellifera*	*Rhus lancia*
			A. tortilis	*Z. mucronata*
Solitair	150–160	150	*A. erioloba*	*Adenolobus garipensis*
(lowland savanna)			*A. kirkii*	*B. albitrunca*
			A. mellifera	*B. foetida*
			A. reficiens	*Catophractes alexandri*
			A. tortilis	*Maytenus linearis*
			D. cinerea	
Sesriem	130	100	*A. albida*	*B. albitrunca*
(lowland savanna)			*A. erioloba*	*B. foetida*
			A. hebeclada	*C. alexandri*
			A. karroo	*Lycium* sp.
			A. tortilis	*Rhigozum trichotomum*
			Parkinsonia africana	*Rhus lancia*
				R. undulata
				Z. mucronata
Swakop River	50	30	*A. albida*	*Euclea pseudebenus*
(dry river plain)			*A. erioloba*	*Salvadora persica*
			Prosopis glandulosa	*Tamarix usneoides*

collected. Each sample represented a separate tree. A total of 24 species and 108 individual trees were sampled, most of which were drought deciduous. All Mimosaceae species under investigation are known to be capable of N_2 fixation (Högberg 1986b, 1990). The non-Mimosaceae include 11 different tree genera all of which are considered not to be capable of N_2 fixation (Högberg 1986b, 1990).

The transect represents a gradient in rainfall extending from about 400 mm average annual rainfall at Windhoek to 30 mm close to the coast (Walter 1964; Table 1). This gradient is associated with variation in temperature, which reaches a maximum about 130 km from the coast (base of the escarpment), and which is lower in the highlands as well as close to the coast. Potential evaporation reaches a maximum of about 3500 mm annually at the base of the escarpment (Besler 1972).

Nitrogen isotope ratios ($\delta^{15}N$ values, Rundel et al. 1988) and nitrogen contents were measured using a Finnigan MAT mass spectrometer (delta D). The method is described in detail by Gebauer and Schulze (1991). $\delta^{13}C$-values were determined according to Osmond et al. (1975).

The contribution of N_2 fixation to the total N content of potentially N_2 fixing trees (N_f) was calculated following the approach of Virginia et al. (1988) as

$$N_f = (\delta^{15}N_0 - \delta^{15}N_f)/(\delta^{15}N_0 - \delta^{15}N_z) \quad (1)$$

in which $\delta^{15}N_0$ is the $\delta^{15}N$ value of non-nitrogen-fixing reference plants, $\delta^{15}N_f$ is the $\delta^{15}N$-value of potentially N_2-fixing plants, and $\delta^{15}N_z$ is the $\delta^{15}N$-value of a nodulated nitrogen-fixing plant growing without access to soil nitrogen. Shearer and Kohl (1989) determined this value as -2‰ for *Prosopis glandulosa,* which was one species also collected in this study (introduced from North America: Palgrave 1983). In this study it was assumed that $\delta^{15}N_z$ has a constant value of -2‰. This is supported by the fact that the lowest $\delta^{15}N$ value measured in this study was -2.25‰ *(Acacia tortilis)*. The calculation was made separately for each location and species along the aridity transect by comparing the $\delta^{15}N$ value of a single Mimosaceae tree with the average $\delta^{15}N$ value of all non-Mimocasaceae trees collected at the same location. Following Gebauer and Schulze (1991), N concentrations are expressed in molequivalents (moleq).

Statistical analysis, by nested ANOVA and regression analysis, used the SAS and SPSS statistical package.

Results and discussion

Average $\delta^{15}N$ values, $\delta^{13}C$ values and N concentrations of all samples differed significantly in potentially N_2-fixing and non-N_2-fixing tree species (Table 2). Averaging all sites, species belonging to the Mimosaceae had a significantly lower $\delta^{15}N$ value than non-Mimosaceae

Table 2. Average $\delta^{15}N$ and $\delta^{13}C$ values and average nitrogen concentrations in leaves of potentially N_2-fixing and non-N_2-fixing tree species (using the SAS General Linear Model). LSD: least significant difference

Mean potential	N_2-fixing	non-N_2-fixing	LSD	*F*-value	*P*
$\delta^{15}N$ value (‰)	4.38	7.38	1.37	18.77	<0.0001
$\delta^{13}C$ value (‰)	−25.49	−24.88	0.49	6.02	0.016
N concentration (μ moleq g^{-1})	1.99	1.75	0.27	5.82	0.017

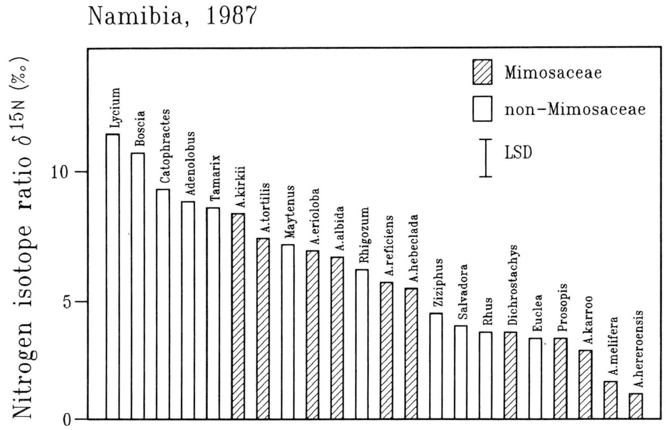

Fig. 1. Variation among species and genera in $\delta^{15}N$-values. Plant names according to Table 1. For the non-Mimosaceae only genera are listed. For *Boscia* and *Rhus* the column indicates the average of two species. *LSD:* least significant difference

species. The contribution of N_2 fixation to total N concentration of the Mimosaceae trees sampled was estimated to average 32%, which is less than estimated by Virginia et al. (1989) for N_2 fixing species of the Sonoran desert. Low $\delta^{15}N$ values were related to higher nitrogen concentrations of leaves in Mimosaceae than in non-Mimosaceae, although the difference was not as large as described by Högberg (1986b). Low $\delta^{15}N$ values were also related to lower intrinsic water use efficiency, as indicated by more negative $\delta^{13}C$-values. The difference in $\delta^{13}C$ values was estimated to be equivalent to a 6 ppm higher internal mesophyll CO_2 concentration in the Mimosaceae (Farquhar et al. 1989). Our interpretation of this result is that N_2-fixing species spend on average more water per mole of carbon uptake than non-N_2-fixing species.

Although average values between N_2-fixing and non-fixing species were significantly different, $\delta^{15}N$-values ranged from +12‰ to +1‰, with the Mimosaceae at the lower end of the scale (Fig. 1). Even species of very similar growth form, e.g. tall trees of *Acacia albida* and *Acacia karoo,* showed considerable differences in $\delta^{15}N$. Rooting depth was suggested as one factor that could cause variations in $\delta^{15}N$-values among species (Virginia et al. 1989), since deep-rooting species may reach soil nitrogen enriched in ^{15}N, while shallow-rooted species may reach soil nitrogen slightly depleted in ^{15}N (Gebauer and Schulze 1991; Virginia et al. 1989). Although we do not know the exact rooting depth of the different species, this did not seem to explain the pattern in the present study (Walter 1964). Differences in leaf longevity among species could affect the $\delta^{15}N$ value. Frequent leaf fall of deciduous species could eventually result in a higher $\delta^{15}N$ compared with evergreen species (Gebauer, unpublished). The present data do not show a consistent pattern in this respect. However, species exhibiting the highest $\delta^{15}N$ enrichment (e.g. *Lycium, Boscia, Catophractes*) were found in habitats with high grazing and burning pressure.

It appears that all processes which lead to some loss

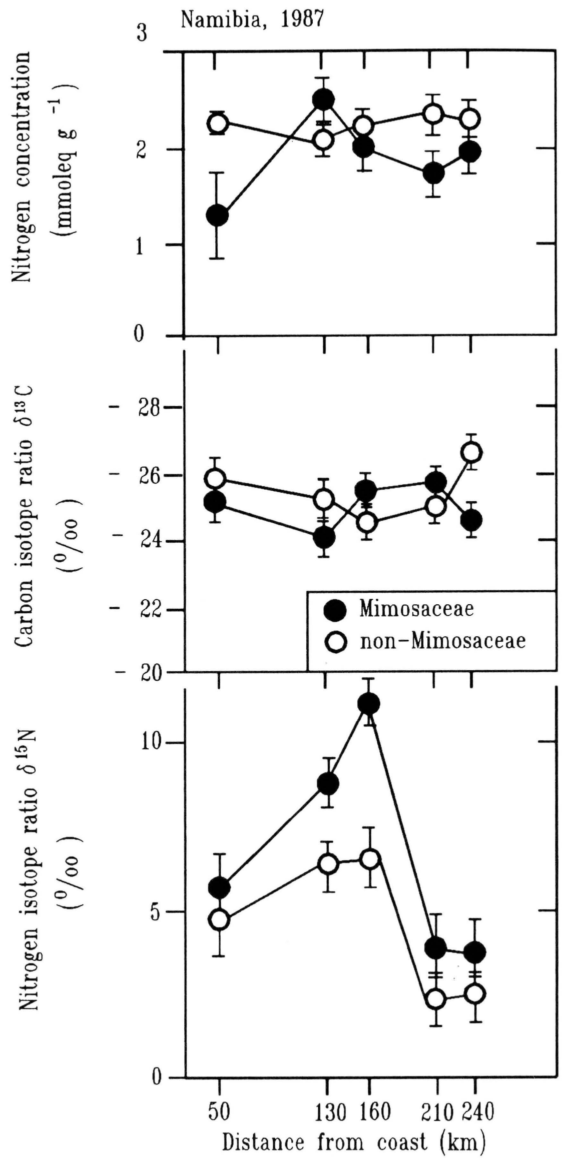

Fig. 2. Variation of $\delta^{15}N$ *(bottom)*, $\delta^{13}C$ *(middle)*, and N concentration *(top)* along an aridity gradient from moist and cool highland savanna to hot and dry lowland savanna and to the coastal fog desert

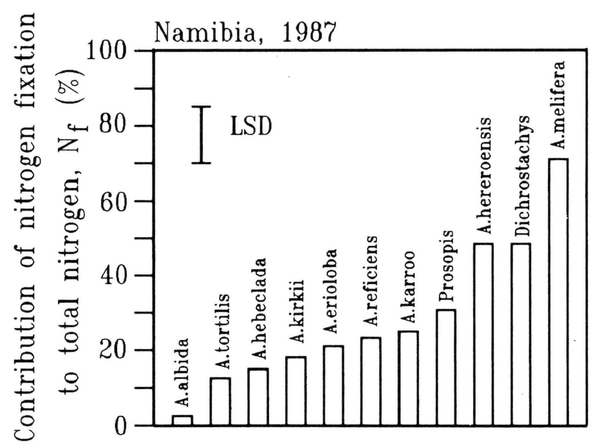

Fig. 3. Variation among species in the contribution of N_2 fixation to the total nitrogen content of leaves (N_f %). The calculation was made separately for each habitat according to Eq. 1

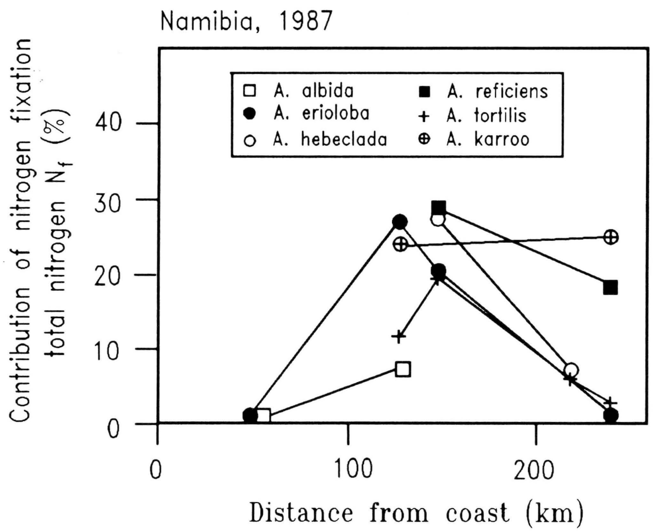

Fig. 4. The contribution of N_2 fixation to the total nitrogen content of leaves (N_f %) of different *Acacia* species along an aridity gradient from the highland savanna to the coast

of nitrogen from the ecosystem increase the background δ^{15}N-value (Gebauer 1991), and partial nitrogen losses, caused for instance by burning, are probably the main factor causing the variation between sites in δ^{15}N-values. The increase in background δ^{15}N in the lowland savanna (Fig. 2) could indicate that there was a considerable loss of nitrogen from the soil. The difference between Mimosaceae and non-Mimosaceae indicates the contribution of N_2 fixation in Mimosaceae at each site. The difference was small in the highland savanna and at the coast and reached a maximum in the lowland savanna. δ^{13}C values and N concentrations did not show the same pattern as δ^{15}N values. In Mimosaceae the N concentration of leaves was remarkably constant at all sites, and δ^{13}C reached most negative values at the dry end of the highland savanna and in the dry lowland savanna. In non-Mimosaceae the N concentration showed a remarkable decrease close to the coast and there was no consistent aridity-related pattern in δ^{13}C values.

The site-specific changes in δ^{15}N values of the non-N_2-fixing reference plants (non-Mimosaceae) makes it necessary to calculate the rate of N_2-fixation separately for each species and habitat. The Mimosaceae showed large species-specific variation in N_f (Fig. 3). For instance, N_f was only 2% in *Acacia albida*, but reached 49% in *A. hereroensis* and *Dichrostachys cinerea* and even 71% in *A. melifera*. In the majority of species N_f ranged between 10% and 30%.

It is more difficult to quantify the habitat-related variation of N_2 fixation, because only few species cover the full range of habitats along the aridity gradient. In species with a broad distribution, such as *Acacia erioloba*, N_f paralleled the pattern of δ^{15}N (Fig. 4, cf. Fig. 2). The increase in N_f from the highland to the lowland savanna was also paralleled by several other *Acacia* species. However, there were also some species which exhibit a very constant rate of N_2 fixation, such as *A. karroo* (Fig. 4). Similar constant N_f values were found in *A. hereroensis* and *Dichrostachys cinerea* (not shown in Fig. 4). *A. albida* was an example in which N_f values also decreased from the lowland savanna to the coastal desert.

Despite all variations between species and habitats, in Mimosaceae N concentration in leaves was constant and independent of δ^{15}N values (Fig. 5). In contrast, N concentrations in leaves of non-Mimosaceae increased significantly with the δ^{15}N-value. The change in δ^{15}N of non-Mimosaceae was not accompanied by a change in

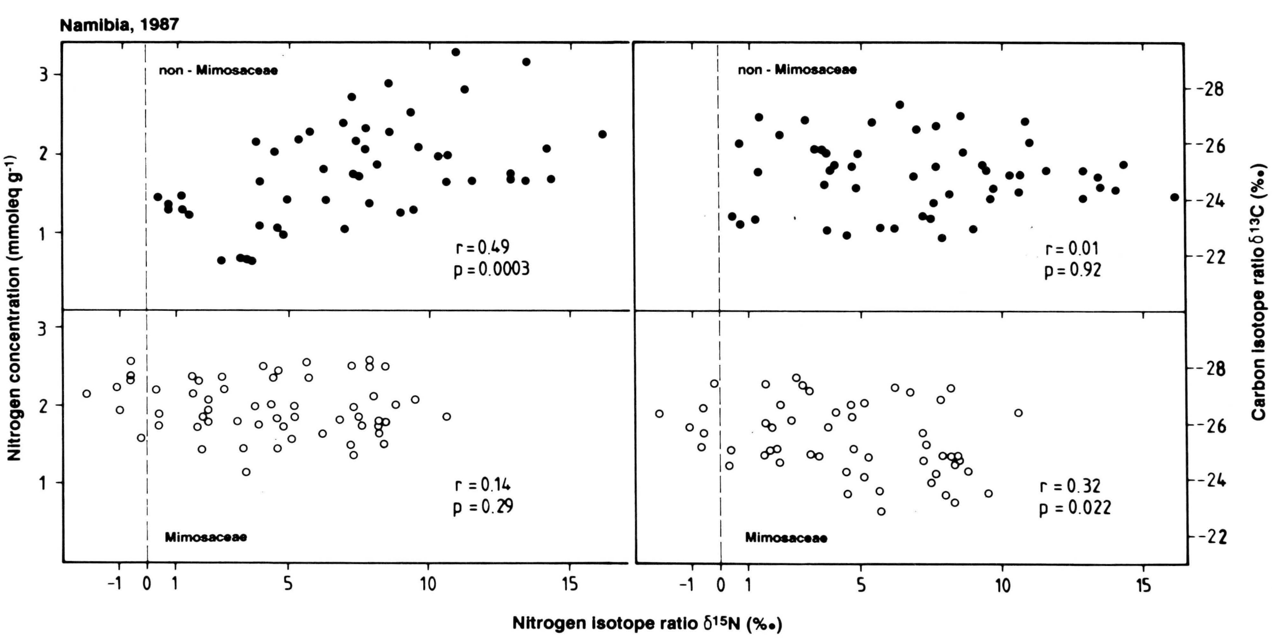

Fig. 5. Relations between nitrogen concentrations *(left)* or δ^{13}C values *(right)* and δ^{15}N values for non-Mimosaceae *(top)* and Mimosaceae *(bottom)*. Each *data point* represents a separate individual tree. *r* indicates the correlation corefficient, *p* the level of significance

$\delta^{13}C$, while in Mimosaceae the $\delta^{13}C$ values decreased with $\delta^{15}N$.

The present study shows that N_2 fixation may be quite important in *Acacia* savannas under certain conditions. However, generally the variation among species in N_f was very large. Despite this it is clear that Mimosaceae and non-Mimosaceae show different physiological responses to increased access to N. Mimosaceae appear to spend more water per unit carbon assimilation, which may be the cost of supplying extra carbohydrates for N_2-fixation.

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