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# Spatial variability in the potential for symbiotic $N_2$ fixation by woody plants in a subtropical savanna ecosystem

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**Summary**

**1.** Root infection by symbiotic $N_2$-fixing *Frankia* and *Rhizobium* strains was quantified in relation to light and soil properties for seedlings of 12 woody species from a subtropical savanna in southern Texas, USA.

**2.** None of four rhamnaceous species nodulated, despite the fact that bioassays with a known actinorhizal species yielded 13 nodules per seedling. *Celtis pallida* (Ulmaceae), *Acacia greggii* and *Acacia berlandieri* (Leguminosae) also failed to nodulate even though field populations of these species were characterized by high (2·7–4·2%) foliar nitrogen concentration.

**3.** Infective rhizobia occurred in all soils studied regardless of soil depth, distance from a host plant or type of plant cover. Plant growth in N-free media and acetylene reduction activity suggested that all nodules were capable of $N_2$-fixation.

**4.** The extent of nodulation varied by species. However, nodulated seedlings were taller, produced more biomass and allocated less biomass to root systems than their non-nodulated counterparts.

**5.** Numbers of nodules on seedlings of *Prosopis glandulosa*, the dominant woody species in this subtropical savanna and throughout the south-western USA, were reduced by low light (15% full sunlight) regardless of soil N level; at medium and full sunlight nodule biomass expressed as a fraction of whole plant biomass decreased with increasing soil N. Nodulation of field-grown *P. glandulosa* appears to be ephemeral, apparently varying with changes in soil moisture.

**6.** Nodulation and $N_2$ fixation among woody legumes in subtropical savannas can occur across a broad range of soil conditions and depths with significant impacts on local and regional N-cycles.

**7.** Field levels of foliar N in species that failed to nodulate in the laboratory were comparable to or greater than those in species capable of nodulation, suggesting that leaf N is not a reliable indicator of $N_2$ fixation.

*Key-words*: *Frankia*, leaf nitrogen, Leguminosae, Rhamnaceae, *Rhizobium*.

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## Introduction

The abundance of woody plants has increased during the past 300 years in arid and semi-arid grassland and savanna ecosystems throughout the world (Archer 1994). In many cases, the encroaching woody species belong to families known to be capable of symbiotic $N_2$ fixation. Consequently, these changes not only affect ecosystem structure but also have the potential for profound modification of biogeochemical attributes (Langkamp, Farnell & Dalling 1982; Schlesinger *et al.* 1990; Schulze *et al.* 1991). Although a few studies have addressed nitrogen fixation by woody plants in arid and semi-arid ecosystems (Shearer *et al.* 1983; Lajtha & Schlesinger 1986; Vitousek & Walker 1989; Högberg 1989; Johnson & Mayeux 1990), our understanding of the extent and significance of this process remains limited.

The historic *Prosopis–Acacia–Andropogon–Setaria* savannas of the Rio Grande Plains of southern Texas (Kuchler 1964) have been largely replaced by subtropical thorn woodland in recent history (Inglis

1964). Hypotheses proposed to account for directional shifts from grass to woody plant domination over the past century have centred around historical enrichment with atmospheric $CO_2$ (Johnson, Polley & Mayeux 1993), intensification of livestock grazing and changes in fire and climatic regimes (Hastings & Turner 1965; Grover & Musick 1990). In heavily grazed ecosystems, losses of soil organic matter, reductions in soil fertility and alterations in physical properties may occur together with loss of vegetative ground cover and erosion (Denevan 1967; Harrington, Oxley & Tongway 1979; Parton *et al.* 1987; Schlesinger *et al.* 1990; Thurow 1991; Walker & Steffen 1993). This could favour $N_2$-fixing woody plants (Bush & van Auken 1989; Cohn, van Auken & Bush 1989; van Auken & Bush 1989; Vitousek & Howarth 1991) and growth forms tolerant of low nutrient conditions (Goldberg 1982).

Shifts from grass to woody plant domination have been documented in southern Texas (Archer 1990, 1994). Of the 40 most important woody plant species in this savanna parkland–woodland system, 18 species belong to families which have been shown to be capable of symbiotic $N_2$ fixation, including 12 species in the Leguminosae (Allen & Allen 1981), four species in the Rhamnaceae (Bond 1983) and two species in the Ulmaceae (Trinick & Hadobas 1988). The ability to fix atmospheric $N_2$ may be an important factor contributing to their recent successful colonization of grassland and savanna ecosystems. Differences in their respective capabilities for $N_2$ fixation may also help to explain patterns of species distribution and abundance across landscapes.

As part of a larger project aimed at understanding vegetation dynamics and the influence of vegetation change on biogeochemical processes in subtropical savannas and thorn woodlands, we conducted studies to (i) assess nodulation capacity for 12 important woody plant species and verify the ability of root nodules to fix atmospheric $N_2$ using the acetylene reduction technique; (ii) quantify soil population levels of nodule-forming bacteria and correlate them with soil characteristics and vegetative cover types; and (iii) quantify the effects of light and soil nitrogen on nodulation and growth of seedlings.

## Study area

Field work was conducted at the Texas Agricultural Experiment Station, La Copita Research Area, located *c.* 15 km from Alice, Texas (27°40′N, 98°12′W) in the eastern Rio Grande Plains of the Tamaulipan Biotic Province (Blair 1950). The climate of the area is subtropical with a mean annual temperature of 22·4 °C and a frost-free period of 289 days. Mean annual rainfall is 680 mm with maxima in May and September. Altitudes within the study area range from 75 to 90 m a.s.l. Soils range from fine sandy loams to sandy clay loams, from slightly acid to alkaline, and include Ustolls (Calcic Chernozems) and Ustalfs (Calcic Luvisols) on uplands, and Aqualfs (Mollic Luvisols) in the lower landscape positions (Scifres & Koerth 1987). See McMahan, Frye & Brown (1984) and Whittaker, Gilbert & Connell (1979) for additional details on vegetation of the region.

Our research was concentrated on sandy loam uplands characterized by a savanna parkland physiognomy consisting of discrete multi-species woody clusters embedded within a continuous matrix of herbaceous vegetation (Archer *et al.* 1988). The woody clusters are typically dominated by the leguminous tree *Prosopis glandulosa* Torrey var. *glandulosa*, with a variety of broad-leaved evergreen and deciduous shrubs in the understorey. Herbaceous vegetation surrounding the clusters consists of $C_4$ grasses (*Bouteloua rigidiseta* (Steudel) A.S. Hitchc., and *Chloris cucullata* Bisch., *Aristida* spp. and *Setaria* spp.) and numerous species of herbaceous dicotyledons.

## Materials and methods

### NODULATION, NODULE ACTIVITY AND FOLIAR NITROGEN

Seven native species from the Leguminosae (*P. glandulosa*, *Acacia farnesiana* (L.) Willd., *A. greggii* A. Gray, *A. rigidula* Benth., *A. berlandieri* Benth., *A. schaffneri* (S. Watson) Herm. and *Eysenhardtia texana* Scheele), four species from the Rhamnaceae (*Colubrina texensis* A. Gray, *Condalia hookeri* M.C. Johnston, *Karwinskia humboldtiana* Zucc., and *Ziziphus obtusifolia* A. Gray), and one species from the Ulmaceae (*Celtis pallida* Torrey) were screened for foliar nitrogen concentration and root nodulation. Taxonomy and taxonomic authorities are based on Correll & Johnston 1979. *P. glandulosa* is a dominant of uplands and intermittent drainages on the site. *A. farnesiana* dominates fine-textured, poorly drained lake bed sites and *A. rigidula* dominates calcareous ridges with shallow, rocky soils. Each of these species is widespread and dominant on many landscapes throughout southern Texas and northern Mexico. The other species are typically subordinate to these in terms of stature and/or density.

Foliar samples were collected in November 1990 from at least three randomly selected plants. For each plant, leaves were collected from three canopy locations (lower, mid and upper), pooled, ground and analysed for total N by combustion-gas chromatography (Carlo Erba Model NA-1500, Fisons Instruments Inc., Danvers, MA). Soil cores (3-cm diameter × 60-cm depth) were collected beneath plants of each species in April 1990 from within 25 cm of the main stem of the target species. They were divided into 0–10, 10–20, 20–35, 35–50 and 50–60-cm depth increments. A soil coring device with a replaceable plastic liner (Forestry Suppliers, Inc., Jackson,

MS) was used to prevent bacterial contamination between cores.

Soil samples collected from beneath each species were mixed with an equal volume of sterile vermiculite and placed in two sterilized plastic containers (4-cm diameter × 20 cm). Seeds of the appropriate species were surface-sterilized in 20% $H_2SO_4$ for 5 minutes, then rinsed five times in sterile distilled water. As an additional check for the presence of *Frankia*, surface-sterilized seeds of two non-native actinorhizal species, *Alnus glutinosa* (L.) Gaertner (Betulaceae) and *Casuarina equisetifolia* L. (Casuarinaceae), were planted in soils associated with *Condalia hookeri* and *Colubrina texensis*, respectively.

Seedlings were grown in a controlled environment chamber set to a 16-hour photoperiod averaging 600 mol $m^{-2}$ $s^{-1}$ of photosynthetically active radiation and 30/26 °C day/night temperature. $CO_2$ concentration in the growth chamber averaged 500 p.p.m. Emerging seedlings were thinned so that the number of seedlings (range of two to six) and seedling size per container were similar for a given species. Seedlings were watered with 1/4-strength N-free Hoagland's solution. Three control containers per species containing sterile vermiculite were planted to verify the absence of *Rhizobium* and *Frankia* strains in the vermiculite and nutrient solution.

Plants were harvested at 8 weeks and soil was washed gently from roots. Shoot height was measured and nodules counted. Nodules were identified with the naked eye and were assumed to be the result of a single infection, though nodules containing multiple rhizobial stains have been documented (Shoushtari & Pepper 1985). Similarly, root nodules have been observed containing multiple *Frankia* strains (Reddell & Bowen 1986) and infection by frankia can occur via different pathways with differential sensitivity to available nitrogen (Kohls & Baker 1989). Roots, shoots and nodules were separated, dried at 70 °C, and weighed.

Whole, nodulated root systems from seedlings of *P. glandulosa*, *Acacia farnesiana*, *A. rigidula*, *A. schaffneri*, *E. texana* and *Alnus glutinosa* were assayed for $N_2$ fixation using acetylene reduction (Hardy, Burns & Holsten 1973). Nodulated root systems (10 per species) were sealed in 50-mL plastic syringes with an acetylene:air atmosphere (1:10 v/v) and incubated for 60 min in the dark at 27 °C. A 5-mL gas sample was removed from the incubation syringes and collected in 7-mL Vacutainers™ (Becton-Dickinson, Rutherford, NJ). Non-nodulated root systems were incubated in an identical manner to quantify background levels of ethylene. Assays were run between 10.00 hours and 14.00 hours to minimize differences associated with diurnal variation. Ethylene was isolated and quantified using flame ionization gas chromatography. The gas chromatograph (Model 300, Antek, Houston, TX) was fitted with a stainless steel column (6·4 mm × 1·3 m) packed with 80/100 mesh Poropak N. Oven temperature was 80 °C and the flow rate of the $N_2$ carrier gas was 30 mL $s^{-1}$.

Analyses of variance for species and soil depth effects on mean number of nodules per seedling were performed with a general linear model procedure of SAS (Parker 1987). Mean values for growth and acetylene reduction rates of nodulated vs. non-nodulated seedlings were compared using Student's *t*-test. Differences in leaf [N] between species classified as nodulating vs. non-nodulating were tested for significance also using Student's *t*-test.

### EDAPHIC INFLUENCES ON NODULATION

To determine how nodulation potential might vary within and between woody plant clusters, we inoculated seedlings with soils collected with cores (3-cm diameter; 0–25, 25–50 and 50–100-cm depth intervals) in May 1990 at the centre of discrete clusters, at the cluster perimeter and from adjoining herbaceous zones 10–15 m from the cluster perimeter. Four woody plant clusters were selected that were approximately circular in shape, 15–30 m in diameter and dominated by 10–30 *P. glandulosa* trees. Typical understorey shrubs included *Zanthoxylum fagara* Sarg., *Condalia hookeri*, *Diospyros texana* Scheele, *Schaefferia cuneifolia* A. Gray, *Z. obtusifolia*, and *Mahonia trifoliolata* (Moricand) Fedde. Soil water content was determined gravimetrically on 25-g subsamples of soil from each depth increment. Soil acidity was quantified on 50-g subsamples in 1:1 soil:distilled water slurry with a pH meter (Model 107, Fisher, Pittsburg, PA). Soil nitrogen was determined using a Carlo Erba NA-1500 elemental analyser.

Sterile plastic containers (4-cm diameter × 20 cm) were filled with approximately 200 mL of sterile vermiculite and planted with surface-sterilized seed (see above) of *P. glandulosa*, *Acacia farnesiana* and *Alnus glutinosa*. The two leguminous species were selected because the study described in the previous section showed them to be capable of nodulation, and they were the dominant woody species within the study area. *A. glutinosa* was used because none of the native rhamnaceous species formed nodules in the experiment described in the previous section and it is relatively promiscuous in terms of the *Frankia* strains that will infect it (Baker 1987).

One week after germination, replicate tubes (four per soil dilution) containing three plants per tube were inoculated with 1 mL sterile distilled water containing 0·0(control), 0·1, 0·01, 0·001, or 0·0001 g of soil. Plants were grown in a controlled environment chamber under conditions identical to those described for the previous experiment. Plants were harvested after 8 weeks, vermiculite was washed gently from roots, and nodules were counted. For most-probable-number (MPN) estimates, tubes with one or more nodules were scored as positive (Vincent 1970). Mean number of nodules per seedling were determined by dividing

the sum of nodules for each of the four dilution series by the total number of seedlings.

An assessment of nodule abundance on field-grown *P. glandulosa* was conducted on 30 April 1992. Soil cores (1·8-cm diameter) were collected from beneath six *P. glandulosa* trees at distances of 0·5, 1·0 and 1·5 m from the bole. Three replicate cores at each distance were collected at 120° intervals around the bole of each tree. Cores were divided into 0–30, 30–60 and 60–100-cm depths, which corresponded to soil volumes of 76·3, 76·3 and 101·8 $cm^3$, respectively. Soils were refrigerated until nodules could be separated by sieving and counted. On the same day as the soil cores were collected, five groups of nodules from three field-grown *P. glandulosa* trees were excavated and assayed for acetylene reduction activity. Nodules were incubated and ethylene production determined as described previously.

The effects of soil depth and location along the cluster centre-to-herbaceous zone transect on numbers of nodules per seedling were evaluated for each species using a general linear model procedure of SAS (Parker 1987). Variation in abundance of field-grown nodules with soil depth and distance from the bole of the tree were evaluated similarly. Simple correlations of numbers of nodules per seedling with MPN estimates and soil properties were determined with SAS.

### EFFECT OF VARYING LIGHT AND NITROGEN LEVEL ON NODULATION

Pots (10-cm diameter × 75 cm) made from PVC tubing were filled with 5·5 L of a non-sterile 1:1:1 mixture of vermiculite, peat and sand, pH 6·6 ± 0·2. Five surface-sterilized seeds of *P. glandulosa*, *Celtis pallida* and *Condalia hookeri* collected from the study site were planted in each pot in June 1993 and thinned to one seedling per pot. At the time of planting, 10 g of soil (0–30 cm depth), collected from beneath conspecific adults growing at the study site, was mixed into the top 10 cm of the potting mixture of each pot. Seedlings were grown outdoors (June through November 1993) under full sunlight and under shade cloth to produce low (15% full sunlight) and moderate (70%) light levels. Pots received weekly additions of 100 mL of full strength Hoagland's solution containing either low (25 p.p.m. N as $NH_4NO_3$), medium (100 p.p.m. N) or high (225 p.p.m. N) nitrogen. Over the course of the experiment the total application of mineral N for the 25 p.p.m. N treatment was comparable to rates of mineralization of N in the top 10 cm of a low-N savanna soil (Bernhard-Reversat 1982). In contrast, levels of mineral N in the 225 p.p.m. N treatment were similar to rates of mineralization under shrub canopies of semi-desert ecosystems (Charley & West 1977). Each light-–nitrogen combination consisted of 12 pots with one seedling per pot. Pots were watered as needed to maintain the soil near field capacity which was monitored by weighing a subsample ($n = 10$) of the pots. After 120 days, seedlings were separated into leaves, stems and roots. Roots were refrigerated until nodules were counted and separated. All tissues were oven-dried at 70 °C and weighed.

## Results

### SPECIES NODULATION CAPACITIES AND NODULE ACTIVITY

Of the 12 native plant species screened, only five leguminous species formed root nodules under laboratory conditions (*Prosopis glandulosa*, *Acacia farnesiana*, *A. rigidula*, *A. schaffneri*, and *Eysenhardtia texana*). Mean number of nodules per seedling ranged from 2·3 for *E. texana* to 43·1 for *P. glandulosa* (Table 1). Mean nodule biomass per seedling ranged from 6·0 mg for *E. texana* to 23·0 mg for *A. farnesiana*. The leguminous *Acacia berlandieri* and *A. greggii* and the ulmaceous *Celtis pallida* did not form nodules. None of the native rhamnaceous species formed root nodules, nor did the actinorhizal *Casuarina equisetifolia* nodulate when grown in soils from beneath the rhamnaceous *Colubrina texensis*. However, the non-native *Alnus glutinosa* did nodulate (12·8 nodules per seedling; nodule biomass of 5·2 mg per seedling), indicating the presence of at least one strain of *Frankia* in the soils associated with *Condalia hookeri*.

Nodulated seedlings were heavier and allocated less biomass to root systems than their control or non-nodulated counterparts (Table 1). Seed biomass, and hence potential levels of stored N for seedling growth varied among species. Biomass of nodulated plants was 10–83 times their seed biomass compared with less than 8 times for non-nodulated control seedlings. The growth of the non-nodulating species was similar to that of non-nodulated control seedlings of the nodulating species.

$N_2$ fixation (nitrogenase activity) in root nodules was confirmed by acetylene reduction assay. Specific nitrogenase activity ($C_2H_4$ evolved per unit dry weight of nodule: $\mu$mol $g^{-1}$ $h^{-1}$) for leguminous seedlings ranged from 25·1 (*E. texana*) to 128·3 (*Acacia farnesiana*) (Table 1). Ethylene production by non-nodulated roots of *P. glandulosa* was 0·8 $\mu$mol $g^{-1}$ $h^{-1}$; control roots of the other species produced no detectable ethylene. Ethylene production by actinorhizal *Alnus glutinosa* (23·3 $\mu$mol $g^{-1}$ $h^{-1}$) was lower than that of leguminous species. Roots of *A. glutinosa* control plants produced no ethylene. Acetylene reduction activity among nodulated plants was significantly correlated with total nodule biomass per seedling ($r = 0·55$, $P < 0·05$), but not with the number of nodules per seedling.

The relationship between number of nodules per seedling and soil depth was species-dependent (Table 2). Among the leguminous shrubs, *P. glandulosa* and *A. farnesiana* were 1–2 in rank-order of nodules per seedling at four of the five depths examined (Fig. 1).

**Table 1.** Nodulation and growth (mean ± SE) of 8-week-old seedlings on a 1:1 host-soil: vermiculite media (or 100% vermiculite for controls) watered with N-free Hoagland's solution. Soils were collected beneath adult conspecifics in a south Texas savanna ecosystem, except exotic *Alnus glutinosa* which was grown on soils from beneath *Condalia hookeri*. Data presented are means across soils from all depths; biomass values are in mg

| Species | *n* | Nodules plant$^{-1}$ | Biomass | | Biomass allocation ratio | | Nitrogenase activity† |
|---|---|---|---|---|---|---|---|
| | | | Nodule | Plant | Plant:seed | Shoot:root | |
| Nodulated species | | | | | | | |
| *Prosopis glandulosa* | 40 | *43·1** ± 2·9 | *19·8* ± 1·1 | *696* ± 30 | *17·3* ± 0·8 | *2·0* ± 0·03 | *61·8* ± 3·7 |
| *Prosopis* control | 27 | 0 ± 0 | 0 ± 0 | 188 ± 7·7 | 4·7 ± 0·2 | 1·3 ± 0·1 | 0·8 ± 0·02 |
| *Acacia farnesiana* | 18 | *20·8* ± 0·8 | *23·0* ± 0·9 | *882* ± 35 | *15·0* ± 0·6 | *2·3* ± 0·07 | *128·3* ± 12·6 |
| *A. farnesiana* control | 7 | 0 ± 0 | 0 ± 0 | 166 ± 2·3 | 2·8 ± 0·04 | 1·1 ± 0·04 | 0 ± 0 |
| *Acacia rigidula*‡ | 10 | *9·7* ± 1·4 | *17·0* ± 2·1 | *294* ± 46 | *10·6* ± 1·6 | *2·0* ± 0·3 | *49·5* ± 9·0 |
| *A. rigidula* control | 6 | 0 ± 0 | 0 ± 0 | 39 ± 12 | 1·4 ± 0·4 | 0·7 ± 0·04 | 0 ± 0 |
| *Acacia schaffneri*‡ | 45 | *13·0* ± 1·2 | *19·5* ± 0·7 | *405* ± 10 | *14·5* ± 0·4 | 1·2 ± 0·01 | *37·4* ± 2·0 |
| *A. schaffneri* control | 3 | 0 ± 0 | 0 ± 0 | 84 ± 12 | 3·0 ± 0·04 | 1·4 ± 0·2 | 0 ± 0 |
| *Eysenhardtia texana* | 10 | 2·3 ± 0·6 | 6·0 ± 1·4 | *79* ± 30 | *23·2* ± 8·8 | 0·8 ± 0·2 | *25·1* ± 0·1 |
| *E. texana* control | 5 | 0 ± 0 | 0 ± 0 | 29 ± 1·3 | 8·5 ± 0·4 | 0·3 ± 0·04 | 0 ± 0 |
| *Alnus glutinosa* | 58 | *12·8* ± 1·6 | *5·2* ± 0·5 | *100* ± 8·9 | *83·3* ± 7·3 | 0·8 ± 0·1 | *23·3* ± 1·3 |
| *A. glutinosa* control | 10 | 0 ± 0 | 0 ± 0 | 8 ± 0·9 | 6·7 ± 0·8 | 0·9 ± 0·06 | 0 ± 0 |
| Non-nodulated species | | | | | | | |
| *Acacia berlandieri*‡ | 50 | 0 ± 0 | 0 ± 0 | 694 ± 27 | 1·3 ± 0·05 | 1·35 ± 0·14 | 0 ± 0 |
| *Acacia greggii* | 20 | 0 ± 0 | 0 ± 0 | 280 ± 14 | 2·2 ± 0·1 | 0·95 ± 0·05 | 0 ± 0 |
| *Celtis pallida*‡ | 30 | 0 ± 0 | 0 ± 0 | 7·5 ± 3·1 | 0·4 ± 0·2 | 5·50 ± 1·97 | 0 ± 0 |
| *Colubrina texensis*‡ | 23 | 0 ± 0 | 0 ± 0 | 171 ± 22 | 3·4 ± 0·4 | 0·59 ± 0·03 | 0 ± 0 |
| *Condalia hookeri*‡ | 48 | 0 ± 0 | 0 ± 0 | 19·4 ± 1·0 | 1·3 ± 0·07 | 1·11 ± 0·09 | 0 ± 0 |
| *Karwinskia humboldtiana*‡ | 17 | 0 ± 0 | 0 ± 0 | 72 ± 18 | 8·3 ± 2·1 | 0·43 ± 0·28 | 0 ± 0 |
| *Ziziphus obtusifolia*‡ | 16 | 0 ± 0 | 0 ± 0 | 69 ± 29 | 3·9 ± 1·6 | 2·11 ± 0·75 | 0 ± 0 |

*Within-species means in *italics* are significantly different from controls at $P < 0·05$.
†Specific nitrogenase activity by acetylene reduction assay as $C_2H_4$ evolved per unit nodule of dry weight ($\mu$mol g$^{-1}$ h$^{-1}$).
‡Nodulation status not previously reported in literature.

Nodulation of *A. farnesiana* and *A. rigidula* seedlings were statistically comparable for soils from all depths. In contrast, nodulation of *A. schaffneri* generally increased with depth, nodulation of *A. glutinosa* decreased markedly with depth, and nodulation of *P. glandulosa* was greatest with soils from 35 to 50-cm depths.

### ESTIMATION OF MPN FOR *RHIZOBIUM* AND *FRANKIA*

Most-probable-number (MPN) values of *Rhizobium* strains infecting *A. farnesiana* seedlings ranged from 170 to 6900 infective cells per gram of soil; with a mean (± SD) of 1408 ± 2161 cells per gram of soil. These values were significantly greater than those obtained for *P. glandulosa* (range = 184–1855; mean = 870 ± 1449). MPN estimates were significantly ($P < 0·05$) correlated with numbers of nodules per seedling (summed over the four dilutions for each soil sample) for both species ($r = 0·83$ for each). Our MPN estimates for *Rhizobium* strains nodulating *P. glandulosa* were in the same range as those reported for *P. glandulosa* plants and soils from a Sonoran Desert site (Virginia, Jenkins & Jarrell 1986). MPN of *Frankia* strains nodulating *Alnus glutinosa* ranged from 0 to 17 infective propagules g$^{-1}$ of soil with an average of 3 ± 6. These values were significantly lower than mean MPN of *Rhizobium* strains nodulating either *P. glandulosa* or *A. farnesiana*. *A. glutinosa* seedlings nodulated with only three of the 12 soils tested. There were no significant main effects or interactions with respect to soil depth or distance from cluster centre on MPN estimates for *Frankia*.

**Table 2.** Analysis of variance for the effects of host species and soil depth on nodulation of 8-week-old seedlings

| Source of variation | Degrees of freedom | *F*-value | *P* |
|---|---|---|---|
| Host | 5 | 31·58 | 0·0001 |
| Depth | 4 | 0·48 | 0·7500 |
| Host × depth | 20 | 3·25 | 0·0001 |

### SPATIAL VARIABILITY AND EDAPHIC INFLUENCE ON NODULATION

Nodulation of *A. farnesiana* seedlings inoculated with soils collected at various depths (to 100 cm) and at various locations relative to the tree bole ranged from 10·5 (herbaceous zones, 0–25 cm depth) to 46·0 nodules per seedling (drip line, 50–100 cm depth: Fig. 2b),

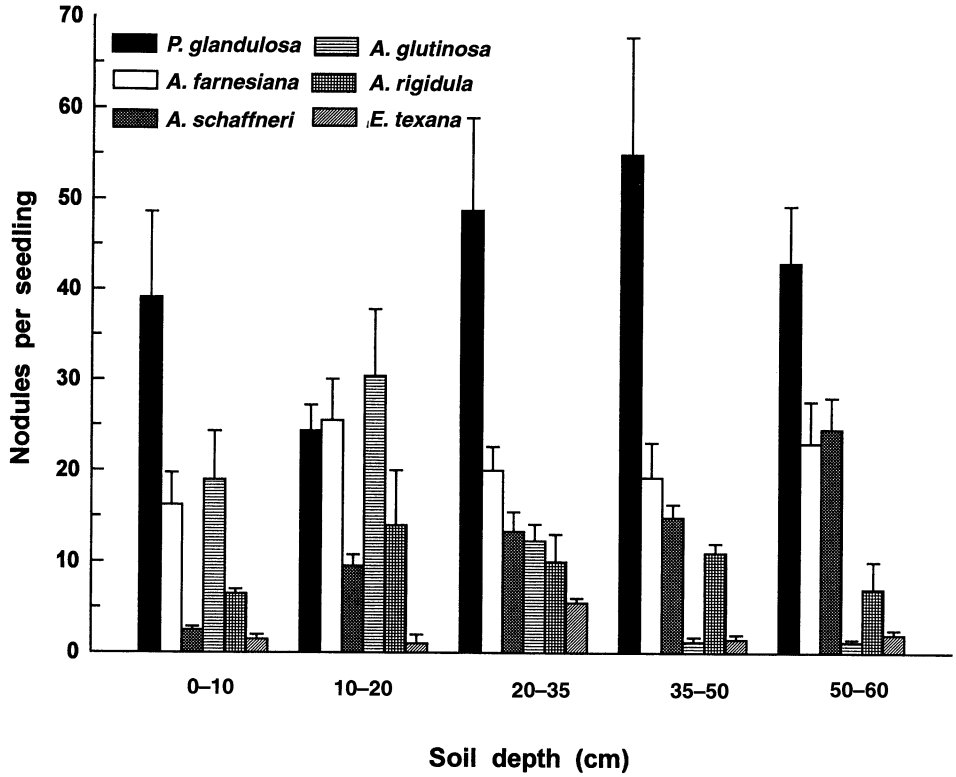


**Fig. 1.** Nodule production by seedlings of *Prosopis, Acacia, Alnus* and *Eysenhardtia* inoculated with soils collected at different depths beneath their canopies (means ± SE).

but did not differ significantly with depth or location (Table 3). The number of nodules on *P. glandulosa* seedlings inoculated with soils from the 50–100 cm depth (16·8 ± 11·3 nodules per seedling) was significantly greater than that for seedlings inoculated with (25–50 cm) soils (10·1 ± 8·2: Fig. 2a). Location relative to tree bole also significantly affected numbers of nodules on *P. glandulosa* (Table 3).

Soil pH ranged from 6·9 ± 1·2–8·0 ± 0·8 (Table 4) and was not correlated with MPN or nodulation potential for either *P. glandulosa* or *A. farnesiana*. Field moisture content of soils at the time of collection ranged from 6·3 ± 4·4% to 10·0 ± 0·4% and was correlated with numbers of nodules on *P. glandulosa* seedlings ($r = 0{\cdot}65$, $P < 0{\cdot}05$), but not with *A. farnesiana* nodule numbers. Mean soil nitrogen ranged from 0·032% at 50–100 cm depths to 0·073% for 0–25 cm depths near tree boles. Soil N was negatively correlated with nodule numbers on *A. farnesiana* seedlings ($r = -0{\cdot}59$, $P < 0{\cdot}099$), but not with numbers of nodules on *P. glandulosa* seedlings. None of these soil characteristics was correlated with nodule numbers on *A. glutinosa* seedlings or MPN estimates for *Frankia* strains.

Nodule abundance on field-grown *P. glandulosa* ranged from 0 to 4·4 nodules per litre of soil with a mean of 1·5 ± 0·4 $L^{-1}$ (Table 5). Nodule abundance was generally greatest 1·5 m from the tree bole and decreased with soil depth. All nodules collected appeared to have begun senescing and no ethylene production was detected. Nodule abundance was low compared with reports for *P. glandulosa* from other regions (0–17 nodules per litre of soil: Johnson & Mayeux 1990). A visual inspection of moist soil samples collected 2 weeks earlier for another study suggested that nodule abundance at the time of our quan-

**Table 3.** Analysis of variance for the effects of location (tree bole vs.dripline vs. herbaceous zone) and depth (0–25 vs. 25–50 vs. 50–100 cm) of soil on nodulation of 8-week-old seedlings of *Prosopis glandulosa* and *Acacia farnesiana*

| Source of variation | Degrees of freedom | *A. farnesiana* | | *P. glandulosa* | |
|---|---|---|---|---|---|
| | | *F* | *P* | *F* | *P* |
| Depth | 2 | 1·13 | 0·37 | 4·09 | 0·02 |
| Location | 2 | 0·29 | 0·76 | 3·76 | 0·03 |
| Depth × location | 4 | 0·70 | 0·61 | 1·78 | 0·14 |

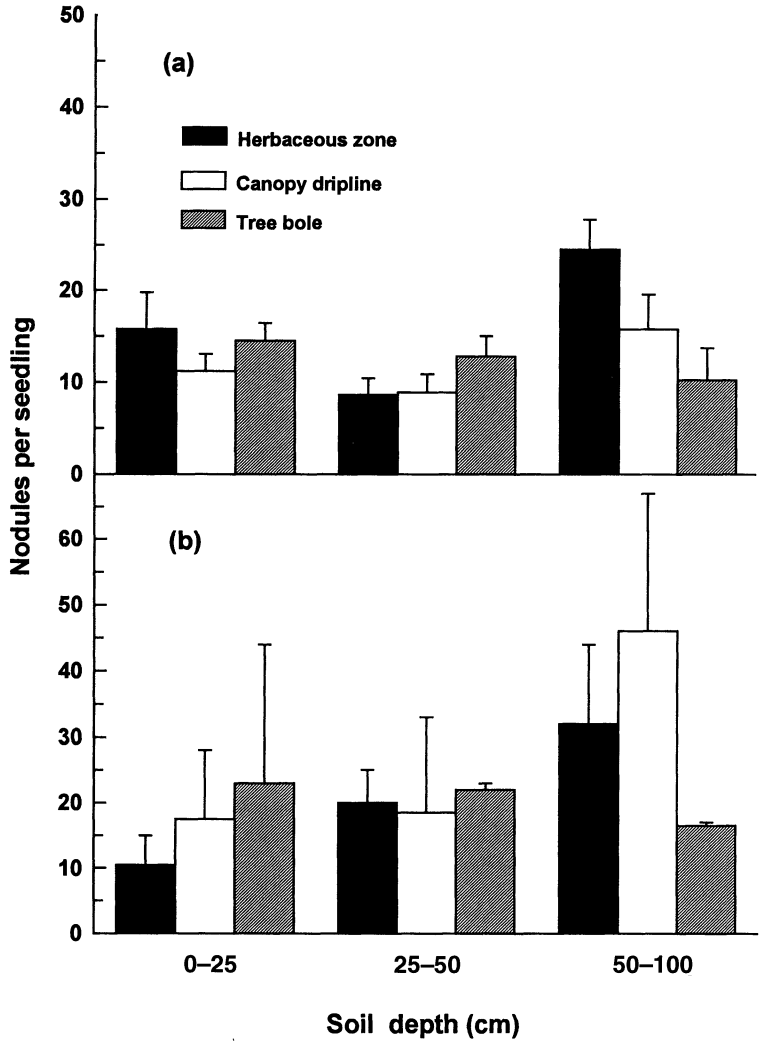


**Fig. 2.** Nodulation (means ± SE) of (a) *Prosopis glandulosa* and (b) *Acacia farnesiana* seedlings inoculated with soils collected at varying depths along transects extending from the tree bole to the canopy dripline and into the adjoining herbaceous zones.

titative sampling had declined markedly, apparently in response to soil drying (e.g. Miettinen *et al.* 1988).

### FOLIAR N

Concentrations of foliar N in leaves collected from plants on the study site ranged from 2·1% to 4·2% (Table 6). Highest foliar [N] occurred among species which did not form nodules in our study (*Acacia berlandieri* = 4·2%; *Celtis pallida* = 3·9%). *Eysenhardtia texana*, which had significantly lower numbers of nodules per seedling in relation to *Prosopis glandulosa* and *A. farnesiana* (Table 1), also had a relatively high foliar [N] of 3·7%. As a group, species classified as nodulated based on our results (Table 1) had foliar [N] similar to those classified as non-nodulated.

### EFFECTS OF LIGHT AND NITROGEN ON NODULATION

Nodulation of *Prosopis glandulosa* occurred under all light and nitrogen combinations (Fig. 3a), but the number of nodules per seedling at 15% full sunlight was less than 15% that of seedlings grown under higher illumination. *Celtis pallida* and *Condalia hookeri* failed to nodulate under any of the treatment combinations. Fertilizer N concentration over the range used in this study had little effect on numbers of nodules per seedling. However, when nodule biomass was expressed as a fraction of total seedling biomass there were significant decreases in relative nodule biomass per seedling with increasing soil N addition under all light levels (Fig. 3b). Maximum mean total seedling biomass occurred in the high light, high N treatments for all species (*C. pallida* = 9·5 g; *C. hookeri* = 8·8 g; *P. glandulosa* = 16·5 g). Total seedling biomass for non-nodulating *C. pallida* and *C. hookeri* was reduced by 68% and 84%, respectively, in the low N treatment compared with the medium N treatment under medium and high light (data not shown). In contrast, total seedling biomass of nodulated *P. glandulosa* seedlings was comparable in the low and medium N treatments under medium and high illumination (Fig. 3c), suggesting that $N_2$ fixation was significantly compensating for low soil mineral N.

**Table 4.** Variation in soil properties (mean ± SD) with depth along a gradient extending from the centre of *Prosopis*-mixed species woody plant clusters to the cluster perimeter and into adjacent herbaceous zones ($n = 4$). Values within rows and within columns were statistically comparable, except for soil moisture at 50–100 cm in the herbaceous zone which exceeded that in the cluster centre

| | Location | | |
|---|---|---|---|
| Depth (cm) | Cluster centre | Cluster perimeter | Herbaceous zone |
| Acidity (pH) | | | |
| 0–25 | 6·9 ± 1·2 | 6·9 ± 0·9 | 7·0 ± 1·0 |
| 25–50 | 7·0 ± 1·2 | 7·4 ± 0·8 | 7·2 ± 0·8 |
| 50–100 | 8·0 ± 0·2 | 7·9 ± 0·2 | 8·0 ± 0·8 |
| Water content (%) | | | |
| 0–25 | 7·7 ± 4·2 | 6·3 ± 4·4 | 8·1 ± 7·0 |
| 25–50 | 7·5 ± 2·6 | 6·5 ± 2·9 | 7·5 ± 3·6 |
| 50–100 | 6·9 ± 0·1 | 7·7 ± 1·4 | 10·0 ± 0·4 |
| Total nitrogen (%) | | | |
| 0–25 | 0·073 ± 0·018 | 0·055 ± 0·011 | 0·057 ± 0·011 |
| 25–50 | 0·044 ± 0·001 | 0·045 ± 0·004 | 0·049 ± 0·011 |
| 50–100 | 0·032 ± 0·004 | 0·033 ± 0·005 | 0·038 ± 0·015 |

**Table 5.** Effect of soil depth and distance from the bole of field-grown *Prosopis glandulosa* trees on *P. glandulosa* nodule frequency on 30 April 1992 (frequency given as number of nodules per unit soil volume, in units of $L^{-1}$ ± SE)

| | Distance from bole (m) | | | |
|---|---|---|---|---|
| Soil depth (cm) | 0·5 | 1·0 | 1·5 | 0–1·5 |
| 0–30 | *2·9* ± 1·3 | 4·4 ± 2·5 | 4·4 ± 2·7 | *3·6* ± 1·1 |
| 30–60 | 0 | 1·0 ± 1·0 | 2·2 ± 2·0 | 0·7 ± 0·5 |
| 60–100 | 0 | 0 | 3·3 ± 0·8* | 0·5 ± 0·3 |
| 0–100 | 0·9 ± 0·4 | 1·6 ± 0·8 | 3·3 ± 1·9 | 1·5 ± 0·4 |

Means in *italics* are significantly greater than for other depths within a distance; means followed by an asterisk are significantly greater than other distances at the same depth, $P < 0·05$.

**Table 6.** Mean (± SD) nitrogen concentration (dry weight basis) in foliage collected from woody plants growing in a south Texas savanna. Species are categorized as nodulated or non-nodulated according to their ability to produce nodules when inoculated with soils from the study site (Table 1)

| Species | N (%) |
|---|---|
| Nodulated | |
| *Prosopis glandulosa* | 2·9 ± 0·45 |
| *Acacia farnesiana* | 3·8 ± 0·38 |
| *A. rigidula* | 2·3 ± 0·17 |
| *Eysenhardtia texana* | 3·7 ± 0·16 |
| Group mean | 3·2 ± 0·71 |
| Non-nodulated | |
| *Acacia berlandieri* | 4·2 ± 0·46 |
| *A. greggii* | 2·7 ± 0·28 |
| *Celtis pallida* | 3·9 ± 0·77 |
| *Colubrina texensis* | 3·1 ± 0·20 |
| *Condalia hookeri* | 2·1 ± 0·17 |
| *Karwinskia humboldtiana* | 2·8 ± 0·06 |
| *Ziziphus obtusifolia* | 2·2 ± 0·11 |
| Group mean | 3·0 ± 0·80 |

## Discussion

Nodule formation on non-native *A. glutinosa* seedlings indicated the presence of viable, infective and effective *Frankia* strains in the soils of the study site, primarily in the upper 35 cm of the soil (Fig. 1). Lack of nodule formation on native rhamnaceous seedlings (*Condalia hookeri*, *Colubrina texensis*, *Karwinskia humboldtiana* and *Zizyphus obtusifolia*) suggests that these species may not be capable of forming nodules under natural or experimental conditions. Seedling size or age does not appear to have been a factor, since seedlings of *Celtis pallida* and *Condalia hookeri* grown for 18 weeks under varying light and nitrogen levels also failed to nodulate. Although actinorhizal and rhizobial infection may be limited by environmental factors such as soil nitrogen and pH (Jenkins, Virginia & Jarrell 1988; McConnell & Bond 1957; Zitzer & Dawson 1992), these should not have been a factor in our study.

*Frankia* strains are spore-forming soil saprophytes and facultative symbionts (Smolander & Sundman 1987; Paschke & Dawson 1992), which may explain

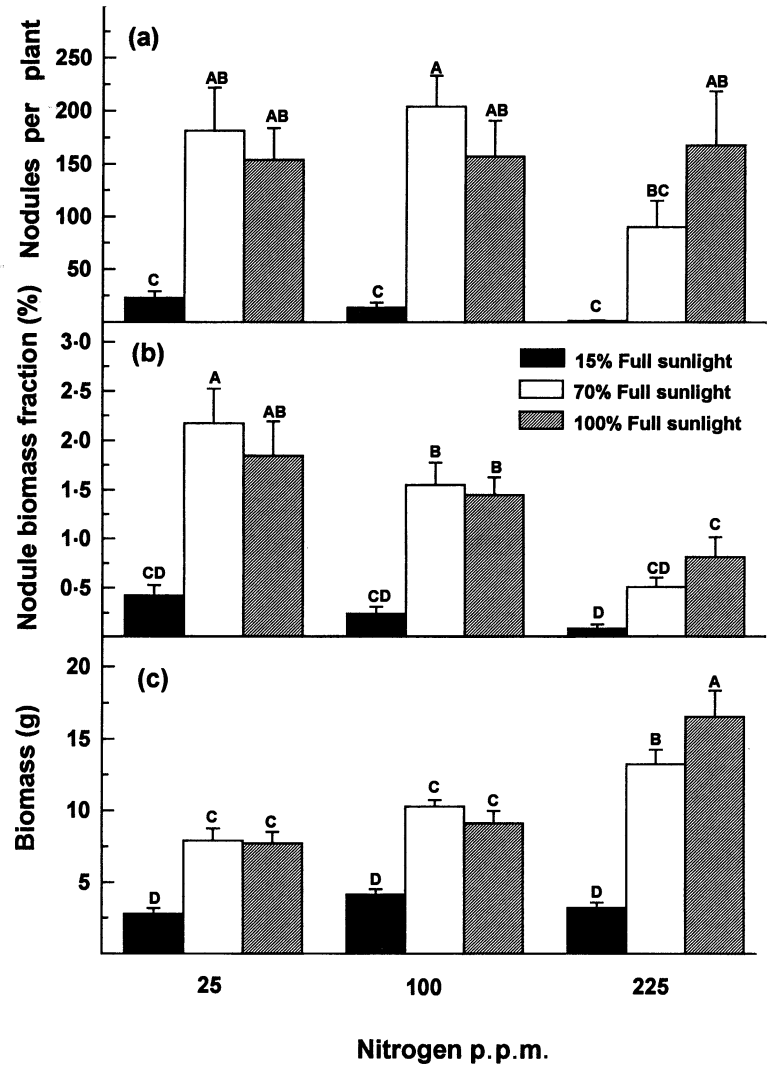


**Fig. 3.** Effects of varying light and nitrogen levels on *Prosopis glandulosa*: (a) nodules per plant; (b) nodules as a fraction of whole plant biomass; and (c) whole plant biomass. Means ± SE are are not significantly different if labelled with the same letter.

their presence and dominance in the surface soils of our study site despite the apparent absence of known host species. *Rhizobium* strains are also facultative symbionts, though populations of host-specific strains are often correlated with the presence of host plants (Woomer, Singleton & Bohlool 1988). However, their ability to survive under adverse soil conditions is strain-specific (Graham 1992), which may account for some of the variation in nodule numbers per seedling and MPN estimates for those host species that did nodulate. The lack of nodule formation on rhamnaceous and ulmaceous species surveyed from this savanna ecosystem may partially explain why leguminous woody species such as *P. glandulosa* and *A. farnesiana* are the more aggressive invaders of low-N soils and why rhamnaceous and ulmaceous shrubs typically appear subsequent to establishment of $N_2$-fixing species (Archer *et al.* 1988).

Foliar N concentration of *C. pallida* at our savanna study site was comparable to or higher than that of *P. glandulosa*, a well-known $N_2$-fixing species (Shearer *et al.* 1983; Lajtha & Schlesinger 1986; Johnson & Mayeux 1990). This fact, coupled with reports of nodulation and $N_2$ fixation by other species in the Ulmaceae (Trinick & Hadobas 1988), led us to anticipate that nodulation might occur. The failure of *C. pallida* to nodulate in this study suggests that factors other than $N_2$ fixation (effective mycorrhizae or its common association with $N_2$-fixing species such as *P. glandulosa*) are more likely explanations for the high tissue N concentrations observed in field plants. Two of the leguminous shrubs examined, *Acacia greggii* and *A. berlandieri*, also failed to nodulate. Lack of nodulation in *A. greggii* has also been noted by Garcia-Moya & McKell (1970), Allen & Allen (1981) and Felker & Clark (1981). Lack of nodulation of *A. berlandieri*, not previously documented, was initially surprising, given the high concentrations of

foliar N in field plants (Table 6). The fact that many of the species which did not nodulate and had foliar N concentrations comparable to or higher than that of plants capable of nodulation indicates that foliar N concentration is not a reliable indicator of $N_2$ fixation in field populations.

Nodulation occurred in five of the native leguminous shrub species and the exotic actinorhizal *Alnus glutinosa*. These nodules produced significant amounts of ethylene (Table 1), indicating that effective *Rhizobium* and *Frankia* strains were present in the soils of our study site (Baker & Torrey 1980; Turk & Keyser 1992). Furthermore, the rates of ethylene production per unit dry weight, at 23–128 $\mu$mol $g^{-1}$ $h^{-1}$ (Table 1), were comparable to those reported for other woody legumes and actinorhizal species (van Kessel *et al.* 1983; Zitzer & Dawson 1989).

High levels of N in surface soils beneath woody plant canopies may produce local spatial patterns, whereby nodulation increases with depth and distance from adult canopies (Virginia *et al.* 1986; Jenkins *et al.* 1988; Jenkins, Virginia & Jarrell 1989; Johnson & Mayeux 1990). In our study potential differences in soil characteristics associated with depth in the profile or distance from centre of woody clusters (Table 4) could not explain the significant differences in numbers of nodules per seedling for *P. glandulosa* (Fig. 2). However, we observed a slight but significant negative correlation between soil N and numbers of nodules on *A. farnesiana* seedlings. Increasing levels of N did not reduce the number of nodules on 18-week-old *P. glandulosa* seedlings (Fig. 3a), but did reduce the nodule fraction of whole seedling biomass: an effect also observed for several *Acacia* species (Sun, Sands & Simpson 1992). This suggests that the levels of soil N encountered in the soils of our study site (Table 4) would be unlikely to prevent infection of the nodulating species by *Rhizobium* strains.

We found no evidence to suggest that vertical or horizontal spatial availability of *Rhizobium* or *Frankia* strains might limit nodule formation in upland soils at this savanna parkland site. Extent of nodule formation under field conditions will depend on light levels, nitrogen availability (Fig. 3a; van Auken & Bush 1989; Lal & Khana 1993) and other factors that influence plant–microbe interactions. Field observations at our site and others (Miettinen *et al.* 1988; Johnson & Mayeux 1990) suggest that nodule formation and persistence is dynamic and ephemeral, and that it fluctuates in response to soil moisture.

Our results add to a growing body of literature which indicate that nodulation in many dryland woody legume species can occur across a broad range of soil characteristics and depths (Virginia *et al.* 1986; Jenkins *et al.* 1988, 1989). The dynamics of nodulation and the extent of $N_2$ fixation under field conditions at our study site awaits further documentation. Such determinations should improve our understanding of the influence of symbiotic $N_2$ fixation on carbon and nitrogen cycling, species interactions, successional processes and responses to disturbance, as well as facilitating the development of sustainable land management in this region.




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## References

Allen, O.N. & Allen, E.K. (1981) *The Leguminosae*. University of Wisconsin Press, Madison.

Archer, S. (1990) Development and stability of grass/woody mosaics in a subtropical savanna parkland, Texas, USA. *Journal of Biogeography*, **17**, 453–462.

Archer, S. (1994) Tree-grass dynamics in a subtropical savanna parkland: reconstructing the past and predicting the future. *Ecoscience*, **2**, 83–99.

Archer, S., Scifres, C.J., Bassham, C.R. & Maggio, R. (1988) Autogenic succession in a subtropical savanna. Conversion of grassland to thorn woodland. *Ecological Monographs*, **58**, 111–127.

Baker, D. (1987) Relationships among pure cultured strains of *Frankia* based on host specificity. *Physiologia Plantarum*, **70**, 245–248.

Baker, D. & Torrey, J.G. (1980) Characterization of an effective actinorhizal microsymbiont, *Frankia* sp. AvcI1 (Actinomycetales). *Canadian Journal of Microbiology*, **26**, 1066–1071.

Bernhard-Reversat, F. (1982) Biogeochemical cycle of nitrogen in a semi-arid savanna. *Oikos*, **38**, 321–332.

Blair, W.F. (1950) The biotic provinces of Texas. *Texas Journal of Science*, **2**, 93–117.

Bond, G. (1983) Taxonomy and distribution of non-legume nitrogen-fixing systems. *Biological Nitrogen-fixation in Forest Ecosystems: Foundation and Application* (eds J. C. Gordon & C. T. Wheeler), pp. 55–87. Martinus Nijhoff/Dr W. Junk, The Hague.

Bush, J.K. & van Auken, O.W. (1989) Soil resource levels and competition between a woody and herbaceous species. *Bulletin of the Torrey Botanical Club*, **116**, 22–30.

Charley, J.L. & West, N.E. (1977) Micro-patterns of nitrogen mineralization activity in soils of some shrub-dominated semi-desert ecosystems of Utah. *Soil Biology and Biochemistry*, **9**, 357–365.

Cohn, E.J., van Auken, O.W. & Bush, J.K. (1989) Competitive interactions between *Cynodon dactylon* and *Acacia smallii* seedlings at different nutrient levels. *American Midland Naturalist*, **121**, 265–272.

Correll, D.S. & Johnston, M.C. (1979) *Manual of the Vascular Plants of Texas*. University of Texas, Dallas.

Denevan, W.M. (1967) Livestock numbers in nineteenth century New Mexico, and the problem of gullying in the southwest. *Annals of the Association of American Geography*, **57**, 691–703.

Felker, P. & Clark, P.R. (1981) Nodulation and nitrogen fixation (acetylene reduction) in desert ironwood (*Olneya tesota*). *Oecologia*, **48**, 292–293.

Garcia-Moya, E. & McKell, C.M. (1970) Contribution of shrubs to the nitrogen economy of a desert wash plant community. *Ecology*, **51**, 81–88.

Goldberg, D.E. (1982) The distribution of evergreen and deciduous trees relative to soil type: an example from the Sierra Madre, Mexico, and a general model. *Ecology*, **63**, 942–951.

Graham, P.H. (1992) Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. *Canadian Journal of Microbiology*, **38**, 475–484.

Grover, H.D. & Musick, H.B. (1990) Shrub land encroachment in southern New Mexico, USA: an analysis of desertification processes in the American Southwest. *Climatic Change*, **17**, 305–330.

Hardy, R.W.F., Burns, R.C. & Holsten, R.D. (1973) Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biology and Biochemistry*, **5**, 47–81.

Harrington, G.N., Oxley, R.E. & Tongway, D.J. (1979) The effects of European settlement and domestic livestock on the biological system in poplar box (*Eucalyptus populnea*) lands. *Australian Rangelands Journal*, **1**, 271–279.

Hastings, J.R. & Turner, R.L. (1965) *The changing mile: an ecological study of vegetation change with time in the lower mile of an arid and semi-arid region.* University of Arizona Press, Tucson.

Högberg, P. (1989) Root symbioses of trees in savannas. *Mineral Nutrition in Tropical Forest and Savanna Ecosystems* (ed. J. Proctor), pp. 212–236. Blackwell Scientific Publications, Oxford.

Inglis, J.M. (1964) *A History of Vegetation on the Rio Grande Plains.* Texas Parks and Wildlife Departmental Bulletin no. 45, Austin, Texas.

Jenkins, M.B., Virginia, R.A. & Jarrell, W.M. (1988) Depth distribution and seasonal populations of mesquite-nodulating rhizobia in warm desert ecosystems. *Soil Science Society of America Journal*, **52**, 1644–1650.

Jenkins, M.B., Virginia, R.A. & Jarrell, W.M. (1989) Ecology of fast-growing and slow-growing mesquite-nodulating rhizobia in Chihuahuan and Sonoran Desert ecosystems. *Soil Science Society of America Journal*, **53**, 543–549.

Johnson, H.B. & Mayeux, H.S. (1990) *Prosopis glandulosa* and the nitrogen balance of rangelands: extent and occurrence of nodulation. *Oecologia*, **84**, 176–185.

Johnson, H.B., Polley, H.W. & Mayeux, H.S. (1993) Increasing $CO_2$ and plant–plant interactions: effects on natural vegetation. *Vegetatio*, **104**, 157–170.

Kohls, S.J. & Baker, D. (1989) Effects of substrate nitrate concentration on symbiotic nodule formation in actinorhizal plants. *Plant and Soil*, **118**, 171–179.

Kuchler, A.W. (1964) *The Potential Natural Vegetation of the Coterminous United States.* American Geographical Society Special Publication no. 36.

Lajtha, K. & Schlesinger, W.H. (1986) Plant response to variations in nitrogen availability in a desert shrubland community. *Biogeochemistry*, **2**, 29–37.

Lal, B. & Khana, S. (1993) Renodulation and nitrogen fixing potential of *Acacia nilotica* inoculated with *Rhizobium* isolates. *Canadian Journal of Microbiology*, **39**, 87–91.

Langkamp, P.J., Farnell, G.K. & Dalling, M.J. (1982) Nutrient cycling in a stand of *Acacia holosericea* A. Cunn. ex G. Don. I. Measurements of precipitation interception, seasonal acetylene reduction, plant growth and nitrogen requirement. *Australian Journal of Botany*, **30**, 87–106.

McMahan, C.A., Frye, R.G. & Brown, K.L. (1984) *The Vegetation Types of Texas.* Texas Parks and Wildlife Department, Austin, Texas.

McConnell, I.T. & Bond, G. (1957) A comparison of the effect of combined nitrogen on nodulation in non-legumes and legumes. *Plant and Soil*, **8**, 378–388.

Miettinen, P., Luukkanen, O., Johansson, S., Eklund, E. & Mulatya, J. (1988) *Rhizobium* nodulation in *Prosopis juliflora* seedlings at different irrigation levels in eastern Kenya. *Plant and Soil*, **112**, 233–238.

Parker, J.C. (1987) *SAS/STAT Guide for Personal Computers, Version 6.* SAS Institute Inc., Cary, NC.

Parton, W.J., Schimel, D.S., Cole, C.V. & Ojima, D.S. (1987) Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal*, **51**, 1173–1179.

Paschke, M.W. & Dawson, J.O. (1992) *Frankia* abundance in soils beneath *Betula nigra* and other non-actinorhizal woody plants. *Acta Oecologia*, **13**, 407–515.

Reddell, P. & Bowen, G.D. (1986) Host *Frankia* specificity within the Casuarinaceae. *Plant and Soil*, **93**, 293–298.

Schlesinger, W.H., Reynolds, J.F., Cunningham, G.L., Huenneke, L.F., Jarrell, W.M., Virginia, R.A. & Whitford, W.G. (1990) Biological feedbacks in global desertification. *Science*, **247**, 1043–1048.

Schulze, E.-D., Gebauer, G., Ziegler, H. & Lange, O.L. (1991) Estimates of nitrogen fixation by trees on an aridity gradient in Namibia. *Oecologia*, **88**, 451–455.

Scifres, C.J. & Koerth, B.H. (1987) *Climate, Soils and Vegetation of the La Copita Research Area.* Texas Agricultural Experiment Station, College Station, Texas.

Shearer, G., Kohl, D.H., Virginia, R.A., Bryan, B.A., Skeeters, J.L., Nilsen, E.T., Sharifi, M.R. & Rundel, P.W. (1983) Estimates of $N_2$-fixation from variation in the natural abundance of $^{15}N$ in Sonoran Desert ecosystems. *Oecologia*, **56**, 365–373.

Shoushtari, N.H. & Pepper, I.L. (1985) Mesquite rhizobia isolated from the Sonoran desert: competitiveness and survival in soil. *Soil Biology and Biochemistry*, **17**, 803–806.

Smolander, A. & Sundman, V. (1987) *Frankia* in acid soils devoid of actinorhizal plants. *Physiologia Plantarum*, **70**, 297–303.

Sun, J.S., Sands, R. & Simpson, R.J. (1992) Genotypic variation in growth and nodulation by seedlings of *Acacia* species. *Forest Ecology and Management*, **55**, 209–223.

Thurow, T.L. (1991) Hydrology and erosion. *Grazing Management: an Ecological Perspective* (eds R. K. Heitschmidt & J. W. Stuth), pp. 141–160. Timber Press, Portland, Oregon.

Trinick, M.J. & Hadobas, P.A. (1988) The biology of the *Parasponia–Bradyrhizobium* symbiosis. *Plant and Soil*, **110**, 177–185.

Turk, D. & Keyser, H.H. (1992) Rhizobia that nodulate tree legumes: specificity of the host for nodulation and effectiveness. *Canadian Journal of Microbiology*, **38**, 451–460.

van Auken, O.W. & Bush, J.K. (1989) *Prosopis glandulosa* growth: influence of nutrients and simulated grazing of *Bouteloua curtipendula*. *Ecology*, **70**, 512–516.

van Kessel, C., Roskoski, J.P., Wood, T. & Montano, J. (1983) $^{15}N_2$ fixation and $H_2$ evolution by six species of tropical leguminous trees. *Plant Physiology*, **72**, 909–910.

Vincent, J.M. (1970) The assessment of nodulation and nitrogen fixation. *Manual for the Practical Study of Root Nodule Bacteria*, pp. 73–104. IBP Handbook No. 15, Blackwell Scientific Publications, Melbourne.

Virginia, R.A., Jenkins, M.B. & Jarrell, W.M. (1986) Depth of root symbiont occurrence in soil. *Biology and Fertility of Soils*, **2**, 127–130.

Vitousek, P.M. & Howarth, R.W. (1991) Nitrogen limitation

on land and in the sea: how can it occur? *Biogeochemistry*, **13**, 87–115.

Vitousek, P.M. & Walker, L.R. (1989) Biological invasion by *Myrica faya* in Hawai'i: plant demography, nitrogen fixation, ecosystem effects. *Ecological Monographs*, **59**, 247–265.

Walker, B.H. & Steffen, W.L. (1993) Rangelands and global change. *Rangeland Journal*, **15**, 95–103.

Whittaker, R.H., Gilbert, L.R. & Connell, J.H. (1979) Analysis of a two-phase pattern in a mesquite grassland, Texas. *Journal of Ecology*, **67**, 935–952.

Woomer, P., Singleton, P.W. & Bohlool, B.B. (1988) Ecological indicators of native rhizobia in tropical soils. *Applied and Environmental Microbiology*, **54**, 1112–1116.

Zitzer, S.F. & Dawson, J.O. (1989) Seasonal changes in nodular nitrogenase activity of *Alnus glutinosa* and *Elaeagnus angustifolia*. *Tree Physiology*, **5**, 185–194.

Zitzer, S.F. & Dawson, J.O. (1992) Soil properties and actinorhizal vegetation influence nodulation of *Alnus glutinosa* and *Elaeagnus angustifolia* by *Frankia*. *Plant and Soil*, **140**, 197–204.

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