



Influence of understory removal, thinning and P fertilization on N₂ fixation in a mature mesquite (*Prosopis glandulosa* var. *glandulosa*) stand

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The natural abundance ¹⁵N/¹⁴N method was used to estimate the influence of silvicultural and P fertilization treatments on N accretion, N₂ fixation and N partitioning among tissues in a mature mesquite *Prosopis glandulosa* var. *glandulosa* stand in Texas. The silvicultural treatments consisted of understory removal, herbicide treatment of brushy resprouts, thinning trees to single stems and 100 kg ha⁻¹ P fertilization. The trees had a mean basal diameter of 17.8 cm with 8 to 35 cm range. The stand was slow growing with the increase in dry matter ranging from 0.465 Mg ha⁻¹ year⁻¹ to 0.701 Mg ha⁻¹ year⁻¹ for the 8 years after the treatments were applied. N accretion after 8 years ranged from 3.1 kg ha⁻¹ year⁻¹ to 4.4 kg ha⁻¹ year⁻¹.

Due to the range in δ¹⁵N of the leaves, twigs, branches and trunk, we used the weighted (by biomass) average δ¹⁵N per tree in calculations of the percent N derived from N₂ fixation (%Ndfa). There was considerable variability in δ¹⁵N of the reference plants, i.e. from 3.3 to 5.9. In contrast there was low variability in the background δ¹⁵N of nearby soils (7.0 ± 1.0). As the total above-ground biomass δ¹⁵N of a grass grown outside the influence of mesquite (7.8 ± 0.58) had the same δ¹⁵N as the soil (7.5 ± 1.0), we used the grass outside the influence of mesquite and the weighted tree mean δ¹⁵N to calculate % of N derived from N₂ fixation.

The decrease in intraspecific competition by thinning multitemed trees to single stemmed trees was the only treatment that significantly ($p = 0.0001$) increased growth. Interspecific competition, i.e. understory removal, did not increase growth. There were no significant differences in total N production or N fixation among treatment means. The most striking result was the highly positive correlation between tree δ¹⁵N and total N per tree and biomass per tree ($R^2 = 0.90$, $F = 164.4$, $df. = 18$, Mean square error (MSE) = 0.155, $p = 0.0001$). This implies that the younger trees colonizing infertile soils relied more heavily on N₂ fixation than larger trees which accumulated 1200 kg ha⁻¹ more N under their canopies. The percentage N derived from N₂ fixation ranged from 63 to 73% in the various treatments. Despite the high percentage of N derived from N₂ fixation, the N₂ fixation of the stand was very low, i.e. 1.98 to 2.80 kg N ha⁻¹ year⁻¹, due to the low growth of the

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stand. We believe that comparisons of the whole tree weighted $\delta^{15}\text{N}$ to background soil $\delta^{15}\text{N}$ provides a more reasonable approach to estimate % N_2 fixation than comparisons of leaves of fixers and reference plants.

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Introduction

Jenny's (1944) classic treatment of soil development clearly showed that temperature influences the amount and kind of vegetation present in an area and is inversely correlated with soil organic matter and nitrogen. Therefore, it is not surprising that the 26% of the continental earth's surface (McGinnies, 1979) that is semi-arid/arid and warm has some of the lowest soil organic matter and soil N concentrations of any ecosystem. For example soil organic N and C contents were 0.027% N and 0.25% C in an arid site of Arizona (Tiedemann & Klemmedson, 1973); 0.011% N and 0.11% C in West Africa (Nicou, 1986); and 0.02% N and 0.10% C in India (Gurbachan *et al.*, 1989).

As reviewed by West & Skujins (1978), Woodmansee (1978) and Felker *et al.* (1980), N inputs into arid ecosystems can be divided into: (1) abiotic sources from rainfall; (2) non-symbiotic N_2 fixers, e.g. *Azotobacter*; (3) cyanobacterial algal lichens; and (4) symbiotic N_2 fixation by herbaceous and woody vegetation. Field studies have found that N input from abiotic sources, non-symbiotic fixers, and cyanobacterial algal crusts are typically less than about 2 kg N ha⁻¹ year⁻¹ whereas leguminous tree N_2 fixation rates of about 35 kg N ha⁻¹ year⁻¹ have been reported (Rundel *et al.*, 1982; Johnson & Mayeux, 1990). We believe the greater N_2 fixation rates reported by leguminous trees are the result of (1) the greater leaf area of the trees (20 g dry matter g⁻¹ N_2 fixed) which can support the energy requirement for N_2 fixation (La Rue & Patterson, 1981) and (2) a combination of greater rooting depth and physiological factors such as osmotic adjustment (Felker & Clark, 1982) which allow them to tolerate greater water stress than herbaceous legumes and lichens.

There can be no doubt that leguminous trees and shrubs, when judged by their percentage of vegetation composition, have been extremely successful in semi-arid regions. *Prosopis* occupies much of the semi-arid regions of south-western United States, Mexico, Caribbean Islands, the coastal deserts of Peru, Ecuador, Chile and all the arid regions of Argentina (Burkart, 1976). In the Sahel and southern Africa, native *Acacia* and naturalized *Prosopis* are the dominant life-forms (Griffith, 1961). In India and Pakistan native and naturalized *Prosopis* occupy much of the arid zones where they provide considerable fuelwood and livestock food (Muthana & Arora, 1983; Shankarnarayan *et al.*, 1987). In Australia hundreds of *Acacia* species dominate the countries extensive semi-arid and arid regions (Thomson *et al.*, 1994).

In spite of the extensive distribution of tree legumes in semi-arid regions, there are very few reports of the measurement of N_2 fixation by the trees in these ecosystems (Shearer *et al.*, 1983; Johnson & Mayeux, 1990; Hamilton *et al.*, 1993; Stock *et al.*, 1995). No doubt the paucity of reports is due to the difficulty in estimating rates of N_2 fixation in trees with root systems that often extend 5 m vertically and horizontally. This makes estimation by the usual method of acetylene reduction and ^{15}N enrichment studies complicated.

Shearer *et al.* (1983) and Rundel *et al.* (1982) pioneered the use of $^{15}\text{N}/^{14}\text{N}$

measurements to estimate the percentage of N₂ fixed by tree legumes in these ecosystems. By additional measurements of the total N produced by the trees, the percentage N₂ fixed, or derived for the atmosphere (%Nd_{fa}), by the tree legumes allowed quantitative estimates of N₂ fixation in these deeply rooted tree legumes. This technique, known as the ¹⁵N natural abundance method, had the advantage of knowing the integrated history of N₂ fixation in legumes. However the technique suffered from the inability to choose a non-fixing reference plant that was using the same N pool as the tree legumes.

Fortunately, semi-quantitative estimates obtained through the ¹⁵N/¹⁴N natural abundance technique and other estimates of N₂ fixation such as nodule abundance (Johnson & Mayeux, 1990) show that tree legumes such as *Prosopis* can fix about 35 kg N ha⁻¹ year⁻¹. This fixation rate is nearly 10-fold greater than all other sources of N into these ecosystems (Loftis & Kurtz, 1980). The method is very advantageous when trying to estimate N₂ fixation at the ecosystem level.

While several measurements of N₂ fixation in tree legumes have been reported, there are no reports of the most important factors that influence N₂ fixation by mature tree legumes at the ecosystem level. It is a common principle of ecology that as one proceeds through the various levels of molecular, tissue, organ, whole plant and ecosystem organization, the control processes change dramatically. There are very great physiological differences between annual legumes and tree legumes in drought stress and salinity tolerance (i.e. *Prosopis* conducts photosynthesis and N₂ fixation at leaf water potentials of 3.8 MPa and growth in 3.2% NaCl). Annual and tree legumes also react differently to the competition arising from coexistence with other species. Therefore, there is little reason to believe that the identical factors which limit N₂ fixation in a field of soybeans will be similar to those at the mature ecosystem level for N₂ fixing trees.

The objective of this study was to gain insights into the relative importance of herbaceous competition, interspecific woody competition, intraspecific woody competition and P fertilization on N₂ fixation and compartmentalization of woody tree legumes at the ecosystem level.

The site chosen for this work has been the subject of earlier investigations. Cornejo-Oveido *et al.* (1992) found that after 3 years the factor most significantly affecting growth of the trees was removal of the understory. East & Felker (1993) found that soils under the canopies of these trees contained 1200 kg ha⁻¹ more N, and 8.3 Mg ha⁻¹ more C than soils outside the canopy. East & Felker (1993) also found that *Panicum maximum* Jacq. var. *trichoglume* Robyns had significantly greater production under the trees than outside the trees. Muthaiya & Felker (1997) found that despite a 3-fold increase in soil P in P fertilized plots 8 years after application, neither leaf tissue P or N were greater in fertilized trees. Patch & Felker (in press) found that after nine growing seasons, trees in P fertilized plots had the greatest growth and significantly greater growth than control plots when growth was normalized for initial basal area.

Methods

Overview

In a mature stand of *Prosopis glandulosa* Torr. var. *glandulosa* estimates were made on the effects of thinning and understory removal on leaf, stem and wood N contents and the natural abundance ¹⁵N/¹⁴N ratios of these compartments (δ¹⁵N). From the δ¹⁵N measurements we estimated the percentage N in the tissues that resulted from N₂ fixation using techniques of Shearer & Kohl (1986). There were approximately 100 trees ha⁻¹ and 220 stems ha⁻¹ with a initial mean basal stem diameter of 16.4 cm,

ranging from 5.7 cm to 40.7 cm. In some cases a single tree had several stems arising from ground level.

Eight years before measurements were made, five silvicultural and P fertilization treatments had been applied in a replicated trial with 30 m by 30 m plots.

Initial biomass was estimated using regression techniques relating basal diameter to biomass. To monitor growth continuously, verniers capable of reading to 0.2 mm in diameter were permanently installed on 20% of the trees. Trees with verniers were stratified according to size classes. New regressions (for each silvicultural or fertilization treatment) were computed to relate the growth of trees with verniers attached to the initial diameter of the same trees. Using these regressions and the initial basal diameter measurements of all trees in the stand, we estimated growth in biomass for all trees in each treatment.

The N dynamics of the stand were determined from intensive measurements on harvest and dry biomass determinations of four entire trees in each of the five treatments (20 trees total). The four trees in each treatment were stratified according to size class (by measuring the basal diameter). Samples from leaves, twigs, branches and main trunk were taken from each of the trees for moisture content, nitrogen content and natural abundance $^{15}\text{N}/^{14}\text{N}$ ratios ($\delta^{15}\text{N}$). Regressions were also computed between branch diameter and tissue dry biomass.

Leaves were also taken from presumed non-fixing reference plants to use in estimating the percentage of N_2 fixed according to Shearer & Kohl (1986).

From the total standing N content and from estimates of the percentage of N resulting from N_2 fixation (%Ndfa), we estimated the N gain (kg ha^{-1}) due to biological N_2 fixation from each treatment.

Study area

The study area was established in 1986 at the Driscoll Ranch (27°32' N and 98°08' W), 32 km west of Kingsville, Texas. The climate is semi-arid sub-tropical. Mean daily maximum temperature exceeded 30°C from April through October. Mean daily temperatures were 35°C for June and 36°C for July and August (United States Department of Commerce, 1965). In August, the warmest month, the mean temperature was 30.2°C (NOAA, 1991). Average yearly rainfall from 1981 to 1991 was 639 mm, with 22.8% occurring during May and June and 29.8% occurring during August and September. September was the wettest month (NOAA, 1991).

The predominant soils were Opelika fine-loamy, mixed, hyperthermic, Mollic Albaqualfs and the Pharr fine-loamy hyperthermic Typic Arguistolls (USDA, 1979).

Experimental design

A randomized complete block design was established in 1986. Five different treatments were randomly assigned to an area of about 3.2 ha on an approximately 30-year-old mesquite natural stand (Cornejo-Oviedo *et al.*, 1992). The treatments were: (1) C: control: vegetation undisturbed; (2) T: thinned: trees were each thinned to one or two main stems; (3) T + UR: trees were thinned and understory vegetation was mechanically removed; (4) T + UR + H: trees were thinned, understory vegetation was mechanically removed and herbicide (GRAZON, ET: 3,5,6 trichloro-2-pyridinyloxyacetic acid) was applied to the resprouts at a rate of 0.72 kg a.i ha^{-1} ; and (5) T + UR + H + P: trees were thinned, understory vegetation was removed, herbicide was sprayed and P fertilizer was applied and left on the surface (triple-superphosphate at a rate of 100 kg ha^{-1}).

Estimation of the above-ground biomass of harvested trees

Four tree size classes were established taking into account the diameter of the trunks at the base (< 14.3 cm, 14.3 to 19.7 cm, 19.71 to 25 cm and > 25 cm). One tree of each size class per treatment was chosen. Trees were harvested with a chain saw at the position where the basal diameter was measured. The tree trunks were cut in segments and the diameters at the sections, as well as the length, were recorded. These measurements were used later to calculate the volume of each segment and of the whole tree trunk. Each segment was immediately weighed with a field scale (Ohaus PL150 with a capacity of 68 kg and a repeatability < 0.01%). Fresh biomass (FW) of the trunk of each tree was determined by the sum of the fresh biomass of all the segments of each tree.

To estimate the trunk dry biomass, four sample disks (approximately 5 cm thick) were cut at different positions from the trunk and weighed. The disks were dried until constant weight at 40°C and the dry biomass was recorded. The volume of trunk segments and sample disks was estimated using the following equation:

$$v_i = \frac{\pi (\text{diam}1/2)^2 + \pi (\text{diam}2/2)^2}{2} \times h \quad (\text{Eqn 1})$$

where diam1 = diameter of the segment at the smaller end, diam2 = diameter of the segment at the larger end, h = length of the segment, and $\pi = 3.14$.

From the sample disk's dry mass (DW) and volume (v_i), the density of each disk was calculated ($D = DW/v_i$). Adding the volumes of all the individual segments, the volume of the entire trunk of the tree was estimated. The percentage of the fresh biomass of the trunk that was dry biomass was calculated for trunks of all the trees of all treatments.

The dry biomass of each tree trunk was estimated from the moisture content of the sample disks taken from it. Since the moisture content changed along the trunk, the percentage to be assigned to a trunk segment was the one of the sample disk whose diameters at both sections were closest to the diameters of the trunk segment. The mean dry biomass percentage for each treatment was calculated.

When the main or secondary trunks were less than 12 cm in diameter, they were considered to be branches. Four of these branches per tree were detached, then immediately weighed to obtain their fresh biomass. The diameter of these and all the rest of the branches of each tree was measured. The branches were dried at 40°C and separated by hand into the different fractions: the main branch-stem, twigs and leaves. The dry biomass of each compartment was obtained separately. Samples were kept in the drying room at 40°C until they were further processed for total N content and $\delta^{15}\text{N}$ analyses.

Regression equations were examined for predicting branch fresh biomass and dry biomass from the diameter at the base of the branch. The \log_{10} -transformed variables were used as they provided the highest correlation coefficient when utilizing the logarithm of the basal diameter as independent variable and the logarithm of the fresh and dry biomass as dependent variables.

The regression equation obtained to estimate the fresh biomass of each branch was:

$$\log_{10} (\text{FW}) = -1.049390 + (2.461818 \times \log_{10} (\text{BD})) \quad (\text{Eqn 2})$$

$(R^2 = 0.84, \text{MSE} = 7.74, N = 91, p = 0.0001),$

where FW is the fresh biomass (kg), and BD is the diameter at the base of the branch (cm).

The regression equation used to estimate the dry biomass of each branch was:

$$\log_{10} (\text{DW}) = -1.252291 + (2.530951 \times \log_{10} (\text{BD})) \quad (\text{Eqn 3})$$

$(R^2 = 0.89, \text{MSE} = 7.74, N = 91, p = 0.0001)$

The estimated dry and fresh biomass of all branches belonging to the same tree, added to the trunk measured fresh biomass and trunk estimated dry biomass, yielded the tree total fresh biomass and dry biomass, respectively.

Estimation of the mesquite stand production through the use of dendrometers

To measure the growth in biomass and volume of the trees, dendrometer bands were permanently mounted around the base of the sample trees in January 1987. The dendrometers were placed as close to the ground as possible, given the forked and multi-stemmed nature of the trees. Twenty trees per treatment were chosen as evenly as possible from among the four replicate plots for dendrometer mounting. The total number of trees in the study site was 416 (95 trees with dendrometers and 371 without dendrometers). The distribution of these trees along the treatments was: 90 for treatment C, 80 for treatment T, 86 for treatment UR + T, 80 for treatment UR + T + H, and 80 for treatment UR + T + H + P.

The dendrometer bands were constructed by modification of Limings's (1957) method. They were made from 2.54-cm wide aluminum, No. 30, gauge strips. The stainless steel springs (Tri-corr Industries Inc., Philadelphia, Penn) were 3.8 cm long by 0.64 cm diameter, with hooks on both ends. A template and scribe, for etching the vernier onto the aluminum bands, was made by the Mechanical Engineering Department of Texas A&M University at Kingsville. The vernier was capable of measuring to 0.25 mm. Dendrometer readings in inches were taken every month during the growing seasons of 1987, 1988 and 1989 (June) by Cornejo-Oviedo *et al.* (1992). These authors developed a mathematical model and a computer program using SAS statistical software (SAS, 1988) to estimate the growth of the trees in each of the treatments. We only took readings in August 1995, i.e. 8 years after initiation.

Above-ground fresh biomass (FW) and volume (V) of 95 trees with dendrometers were estimated after eight growing seasons, through the use of regression equations developed by El Fadl *et al.* (1989). The equations utilized were:

$$\log_{10} (\text{FW}) = 3.82711138 + 1.05238764 \times \log_{10} (\text{BA}) \quad (\text{Eqn 4})$$

$$\log_{10} (\text{V}) = 0.71756102 + 1.27342367 \times \log_{10} (\text{BA}) \quad (\text{Eqn 5})$$

Cornejo-Oviedo *et al.* (1992) developed a model to predict biomass production for all trees in each treatment using five regression equations (one for each treatment) that predicted tree growth in biomass and volume from the initial trunk basal diameter. In this paper, 10 new regression equations, to account for the growth in fresh biomass and volume after 8 years, were developed (Table 1).

The model predicts the fresh biomass and volume increment from the initial trunk basal area of each tree for each silvicultural treatment. The ability of the five regressions (one for each treatment) to predict was determined through the use of the Press statistics (SAS, 1988). These regression equations were used to estimate the growth in fresh biomass of all the trees of the study area.

Given the 1089 m² plot⁻¹, and the distribution of the trees, the fresh biomass production per ha was calculated. An analysis of variance was used to identify treatment differences in fresh biomass production (absolute value) and percentage growth. From the percentages of dry biomass estimated for the 20 harvested trees (four per treatment), we estimated the dry biomass of the stand.

Table 1. Regression equations for the growth in fresh biomass (kg) and volume (m³) of mesquite trees 8 years after application of five management treatments

Treatment*	N	Equation†	R ²	MSE	Press
Control	22	log 10 FW = 2.526908 + 0.84346 (log 10 BAI) log 10 V = -0.497390 + 1.062389 (log 10 BAI)	0.51 0.60	507.4 0.0002	13407.9 0.00445
Thinned	16	log 10 FW = 2.505779 + 0.678590 (log 10 BAI) log 10 V = -0.525739 + 0.890898 (log 10 BAI)	0.26 0.41	155.6 0.00003	2718.1 0.00005
UR+T	20	log 10 FW = 2.211605 + 0.451379 (log 10 BAI) log 10 V = -0.822056 + 0.661245 (log 10 BAI)	0.43 0.53	329.7 0.0001	10517.1 4196.6
UR+T+H	19	log 10 FW = 2.744469 + 0.861549 (log 10 BAI) log 10 V = -0.281348 + 1.078024 (log 10 BAI)	0.56 0.99	196.7 0.00004	4081.9 4196.6
UR+T+H+P	18	log 10 FW = 2.865309 + 0.937097 (log 10 BAI) log 10 V = -0.157593 + 1.155141 (log 10 BAI)	0.48 0.58	393.9 0.00009	7631.8 0.00174

*T = thinned; UR = understory vegetation removed; H = herbicide applied; P = P-fertilizer applied.

†FW = fresh biomass after 8 years; V = volume after 8 years.

R² = coefficient of determination.

Estimation of the total N and biologically fixed N₂ fraction

Dry samples of the trunk, branches, twigs and leaves of one tree of each size class per treatment were ground in a Wiley mill to pass a 0.4 mm sieve. The ground material was analysed at the Isotope Services, INC Laboratory in Los Alamos, New Mexico (329 Potrillo Dr., Los Alamos, NM 87544). The $\delta^{15}\text{N}$ (‰) of all tissue samples was measured. The reproducibility error of the data (two repetitions of the same sample) was always less than 2‰. Sample portions (from 4 to 20 mg of dry matter depending on the tissue being analysed), in duplicate, were weighed into tin capsules using an automated Sartorius micro-balance. The samples were burned in an oxygen pulse in a combustion tube heated to 1018°C. Their isotopic peaks at mass-28 (for ^{14}N), mass-29 ($^{14}\text{N} + ^{15}\text{N}$) and mass-30 ($^{14}\text{N}^{16}\text{O}$) were measured and compared to a reference gas (acetalinide, OAS b2000/catalog #5780 from Micro-analysis). The composition of the reference gas was 10.36% N, 71.09% C, 6.71% H and 11.84% O. The sum of all the N isotopic fractions yields the total N present in the sample. Tree total N (kg) was estimated from summing the products of the dry biomass of each tissue portion (kg) multiplied by the total N percentage of each tissue.

Estimation of the N accretion in the ecosystem

Knowing the productivity of mesquite trees in this ecosystem, i.e. the kg of biomass produced per ha per 8 years for the different treatments, the total amounts of N in the different tissues and in the whole trees were calculated. Extrapolating the percentage of dry biomass of each fraction obtained from the harvested trees to the total dry biomass produced by the stand after 8 years, total N produced per treatment was calculated. The percentage of that N that was biologically fixed (%Ndfa) was also estimated by using the expression (Shearer & Kohl, 1986):

$$\%Ndfa = \frac{\delta^{15}\text{N}_r - \delta^{15}\text{N}_p}{\delta^{15}\text{N}_r - \delta^{15}\text{N}_a} \times 100 \quad (\text{Eqn 6})$$

where $\delta^{15}\text{N}_r$ is the value of non-fixing plants (reference plants), $\delta^{15}\text{N}_p$ is the value of fixing plants in the field (problem individuals), and $\delta^{15}\text{N}_a$ is the $\delta^{15}\text{N}$ value of leaves of young *P. glandulosa* grown hydroponically with N-free nutrient medium over 2 years. This value was estimated to be -1.7‰ (Shearer & Kohl, 1986).

Results

The standing fresh and dry biomass in Table 2 illustrate the effect of thinning in this stand. As might be expected after thinning, the control had greater initial biomass than all the other treatments. Yet after 8 years, some thinned treatments had greater absolute growth rates than the control treatments. While we attempted to locate a uniform stand for this trial, there was an approximate 25% difference in standing biomass among the treatments (Cornejo-Oviedo *et al.*, 1992). Thus while there were no significant ($p = 0.3189$, Table 3) differences in absolute growth for the five treatments, there were very highly significant ($p = 0.0001$) differences in growth calculated as a percentage of original standing biomass between the control and the thinned treatments.

The major influence on growth was the thinning treatment. While the understory removal plus thinning treatment had the greatest absolute growth, it was not significantly ($p = 0.3189$) different from the control or other thinned treatments. Despite a significant 3-fold increase in soil available P in fertilized treatments over the

Table 2. Estimated mean standing biomass and growth 8 years after five silviculture and P fertilization treatments were applied

Treatment*	N	Initial fresh biomass [†] (kg ha ⁻¹)	FWT growth [‡] (kg ha ⁻¹)	Initial DWT (kg ha ⁻¹)	Growth DWT (kg ha ⁻¹)	DWT Growth/ initial (%)
C	4	47200	4800	40200	4060	10.27 ^a
T	4	29200	5130	24900	4390	18.07 ^b
UR+T	4	34600	6480	30100	5610	19.48 ^b
UR+T+H	4	29000	4780	24500	4960	16.83 ^b
UR+T+H+P	4	27800	4730	21700	3720	16.60 ^b

Values are the means of four blocks per treatment.

*C = control; T = thinning; UR = understory vegetation removal; H = herbicide treatment of brush resprouts; P = P fertilization.

DWT = dry biomass; FWT = fresh biomass.

[†]No significant difference between the treatment means ($F = 3.02$, $p = 0.0613$, $df. = 4, 12$, $MSE = 1041522211$).

[‡]No significant difference between the treatment means ($F = 1.32$, $p = 0.3189$, $df. = 4, 12$, $MSE = 8760049$).

Any two treatment means with the same letter do not differ ($p < 0.05$) using Tukey's HSD test.

control, foliage P in the P fertilized treatment was not significantly greater than the control (Muthaiya & Felker, 1996). The P fertilization treatment had no apparent effect on growth. Treatments with herbicide application to control shrub resprout after understory removal seemed to have less growth than the comparable treatment without herbicide application. The standing fresh biomass of about 35 Mg ha⁻¹ for the understory vegetation removal plus thinning treatment compares favorably to the biomass of 4.09 Mg ha⁻¹, 19.39 Mg ha⁻¹ and 36.08 Mg ha⁻¹ reported by Whisenant & Burzlaff (1978) for shallow land, deep upland and bottom land sites, respectively. This biomass is substantially greater than the 13.97 Mg ha⁻¹ standing biomass reported for a California desert site by Sharifi *et al.* (1982) in which N₂ fixation was also estimated by ¹⁵N/¹⁴N natural abundance technique.

In a survey of many Texas sites to determine volume/stand density relations for *Prosopis*, Felker *et al.* (1990) found the standing fresh biomass for many dense stands to be in the 50 to 70 Mg ha⁻¹ range.

In contrast to estimates from natural stands, *Prosopis* plantations may have much greater biomass productivity. Only 30 km away from this site, a non-irrigated but intensively managed *Prosopis alba* Griseb. plantation had a standing dry biomass of 39.3 Mg ha⁻¹ after only 3 years on a 3 m by 3 m spacing (Felker *et al.*, 1989). In a minimally managed progeny trial of Texas native *Prosopis glandulosa* var. *glandulosa*,

Table 3. Analysis of variance for treatment differences in growth (absolute value) and growth as a percentage of the initial fresh biomass (% Growth)

Source	df.	Growth (kg ha ⁻¹)		% Growth	
		MS	p value	MS*	p value
Treat	4	87600494	0.3189	0.04055765	0.0001
Block	3	12556471	0.1077	0.01098040	0.0089
Error	12	19960652		0.00712392	

The design is a randomized complete block design with four blocks and five treatments (see Table 1).

*Mean square (MS) reported for Arcsin transformed % Growth.

Table 4. Dry matter percentages corresponding to the different tissues of mesquite trees subjected to management treatments (N = 4)

Treatment*	Percentage of the tree total dry matter							
	Leaves		Twigs		Branches		Wood	
	Mean†	S.D.	Mean‡	S.D.	Mean§	S.D.	Mean¶	S.D.
C	2.73	(0.38)	14.39	(4.90)	11.27	(3.23)	71.67	(7.36)
T	2.75	(0.66)	14.62	(4.83)	9.45	(3.06)	73.27	(6.98)
UR+T	3.68	(0.70)	14.56	(2.65)	8.58	(5.01)	72.42	(6.20)
UR+T+H	3.16	(2.91)	11.55	(4.14)	10.39	(7.50)	74.84	(1.42)
UR+T+H+P	4.11	(1.45)	16.67	(3.97)	12.45	(5.89)	66.77	(9.70)

Four trees per treatment were harvested between 8 September and 6 October 1993.

*C = control; T = thinning; UR = understory vegetation removal; P = P fertilization.

† $F = 0.62$; df. = 4, 15; MSE = 2.33; $p = 0.6540$.

‡ $F = 0.76$; df. = 4, 15; MSE = 17.45; $p = 0.5650$.

§ $F = 0.34$; df. = 4, 15; MSE = 27.17; $p = 0.8490$.

¶ $F = 0.42$; df. = 4, 15; MSE = 87.37; $p = 0.7885$.

the mean dry biomass yield of all nine families was 69 Mg ha⁻¹ after 10 years, for an annual growth increment of 6.9 Mg ha⁻¹ (Duff *et al.*, 1994).

Thus our standing biomass and growth estimations compare favorably to other *Prosopis* mature stands, but these figures are nearly 10-fold lower than productivities obtained with similar genetic stock in plantations.

While it was surprising that the P fertilization treatment did not give any growth advantage, there appears to be positive benefit of the fertilization as can be seen by the enhanced percentage of leaves and twigs in Table 4. The tree dry biomass for the treatment containing P fertilization had greater partitioning into leaves (4.11%) and twigs (16.67%) than all the other treatments, although these differences were not significant ($p < 0.05$). In all treatments except the P fertilization treatment, the main trunk wood comprised 70% of the standing biomass (Table 4). The initial, final N and growth in N for each of the fractions (Table 5) illustrates that by far the greatest annual increase in N occurs in the trunk wood followed by the twigs. The branches and leaves follow in decreasing order of demand for total N. The trees were harvested in late summer. This partitioning of N may change if the trees are harvested at another time of year.

The greatest leaf increase in N occurred in the understory removal plus thinning treatment, but if the N growth were normalized for equivalent initial stand biomass, the P fertilization treatment and the understory removal plus thinning treatment would be equivalent. The increment in total N over the 8-year period ranged from 24.6 to 35.41 kg ha⁻¹. There were no significant ($p < 0.05$) differences among the treatments regarding N production.

A comparison of the partitioning of N among the tissues, percentage N kg⁻¹ dry biomass, and $\delta^{15}\text{N}$ in the different tissues of mesquite is presented in Fig. 1. N concentration is greatest in the leaves, followed by the twigs, branches and wood, and there is very little difference in N concentration among the various treatments. In contrast, the total N allocated to the various tissues occurred in nearly the reverse order, with the wood having the greatest total N and the leaves having the least total N. A major difference in the order is that twigs contain the second greatest pool of N. The partitioning of N in this study is considerably different than the study of Rundel *et al.* (1982) for Californian mesquite growing phreatophytically. These workers found much greater partitioning of N in the branches (47%) and leaves (20%) than we observed in these compartments (approximately 17% and 10% for the treatment and

Table 5. N productivity at initial and final times, and N increment after 8 years since treatments were applied

Treatment*	Leaves total N (kg ha ⁻¹)		Twigs total N (kg ha ⁻¹)		Branches total N (kg ha ⁻¹)		Wood total N (kg ha ⁻¹)		Total N	
	Initial	Final	Initial†	Final	Initial†	Final†	Initial	Final	Growth	Growth (kg)
C	32.37	35.66	79.26	87.34	42.29†	46.59†	91.48	100.76	4.30	9.30
T	19.70	23.15	51.94	61.06	18.2	21.4	58.66	68.96	3.20	10.30
UR+T	29.99	35.60	61.75	73.29	29.56	35.11	68.09	80.82	5.53	12.73
UR+T+H	26.64	31.01	35.79†	41.67†	22.42	26.11	64.89	75.55	3.69	10.66
UR+T+H+P	26.82	30.22	49.09	57.46	23.06	27.02	50.97	59.65	3.94	8.68

*C = control; T = thinning; UR = understory vegetation removal; H = herbicide applied; P = P fertilization.

†Treatment mean is significantly different (p<0.06) using Tukey's HSD test.

control, respectively). Rundel *et al.* (1982) found less N in the main stem (20%) than we observed (approximately 40%). Their trees had tapped into a permanent ground-water source and also had a much greater annual productivity of 3700 kg dry matter.ha⁻¹.year⁻¹ than our figure of about 600 kg.ha⁻¹.year⁻¹. With only 34% coverage of the ground surface in the California study and no other woody or herbaceous competition, the California *Prosopis* had full access to sunlight on all sides and thus with unlimited water could also produce more leaf matter.

The $\delta^{15}\text{N}$ in the various tissues of our study area is in general higher (less % N₂ fixed) than in the study of Shearer *et al.* (1983). For example their $\delta^{15}\text{N}$ values were

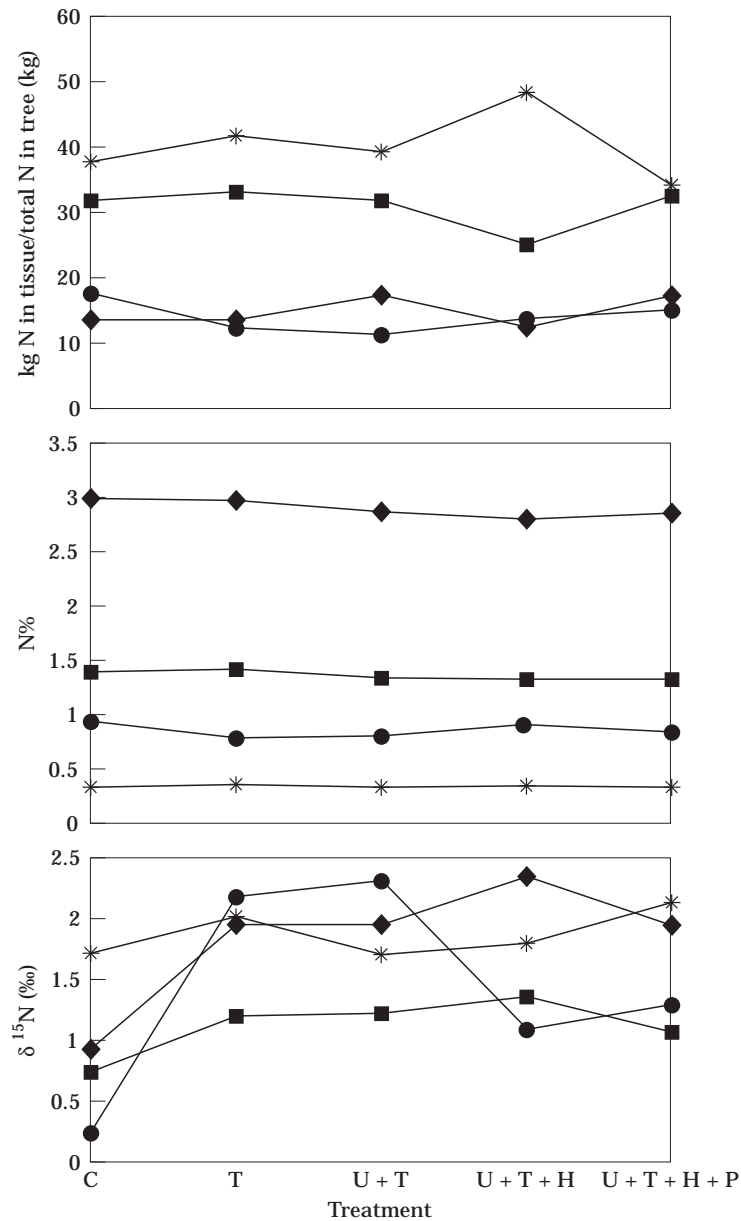


Figure 1. Relative N (%), total N (%) and $\delta^{15}\text{N}$ in different tissues of mesquite. Values are the mean of four trees per treatment. (●) = branches; (◆) = leaves; (■) = twigs; (*) = wood.

1.2 for leaves, 0.9 for twigs, 0.1 for branches and -3.5 for trunk wood. In contrast, except for trees in the control plots, all of our $\delta^{15}\text{N}$ values were greater than 1. The control trees had lower $\delta^{15}\text{N}$ values for leaves, twigs, and branches than all other treatments suggesting that they were obtaining a greater percentage of their N from biological N_2 fixation than trees in the other treatments.

To examine all possible relationships between tree biomass, N concentrations in the tissues and N_2 fixation ($\delta^{15}\text{N}$) a correlation matrix was created (Table 6). It is important to realize that the higher the $\delta^{15}\text{N}$ value, the lower is the percentage of N_2 fixed, e.g. Eqn (6) in materials and methods. Thus it is only negative correlations with $\delta^{15}\text{N}$ that indicate high N_2 fixation. As might be expected, leaf $\delta^{15}\text{N}$ was significantly correlated with twig ($r = 0.497, p = 0.026, N = 20$), branch ($r = 0.532, p = 0.016, N = 20$) and wood ($r = 0.495, p = 0.026, N = 20$) $\delta^{15}\text{N}$. The only negative correlation was between $\delta^{15}\text{N}$ of the branches and the N concentration of the branches ($p = 0.01, r = -0.70, N = 20$). Thus it would seem that the branches are the most likely first site for translocation of fixed N_2 from the roots at this time of the year. The tree total dry biomass in kg was found to be significantly correlated with the N concentration in the wood ($r = 0.492, p = 0.028, N = 20$), the tree N concentration ($r = 0.99, p = 0.0001, N = 20$) and tree $\delta^{15}\text{N}$ ($r = 0.949, p = 0.0001, N = 20$). The N concentration in the wood was significantly correlated with the N concentration of the entire tree ($r = 0.50, p = 0.03, N = 20$) and the $\delta^{15}\text{N}$ of the twigs ($r = 0.58, p = 0.03, N = 20$).

Certainly the most striking result was the highly significant ($r = 0.949, p = 0.0001, N = 20$) correlation between tree $\delta^{15}\text{N}$ and tree biomass. The tree $\delta^{15}\text{N}$ was the

Table 6. Pearson's product moment correlation coefficient for tissue total N percentage and $\delta^{15}\text{N}$, tree biomass, tree total N percentage and tree $\delta^{15}\text{N}$

	Tree DWT	$\delta^{15}\text{N}$ Leaf	$\delta^{15}\text{N}$ Twig	$\delta^{15}\text{N}$ Branch	$\delta^{15}\text{N}$ Wood	Leaf N%	Twig N%	Branch N%	Wood N%	Tree N%
$\delta^{15}\text{N}$	0.121									
Leaf	0.609*									
$\delta^{15}\text{N}$	0.208	0.50								
Twig	0.379	0.03								
$\delta^{15}\text{N}$	0.120	0.53	0.30							
Branch	0.615	0.02	0.20							
$\delta^{15}\text{N}$	0.203	0.50	0.01	0.39						
Wood	0.390	0.03	0.98	0.09						
Leaf	0.239	-0.21	-0.02	0.19	0.13					
N%	0.311	0.38	0.93	0.42	0.59					
Twig	0.209	-0.08	0.02	-0.13	0.04	0.08				
N%	0.376	0.73	0.94	0.60	0.88	0.73				
Branch	0.202	-0.22	-0.03	-0.70	-0.16	-0.19	0.50			
N%	0.392	0.35	0.89	0.001	0.51	0.43	0.02			
Wood	0.492	0.39	0.58	0.05	-0.02	-0.26	0.12	0.23		
N%	0.028	0.09	0.008	0.85	0.94	0.27	0.62	0.33		
Tree	0.994	0.13	0.20	0.10	0.20	0.22	0.23	0.24	0.50	
N%	0.0001	0.59	0.41	0.68	0.40	0.36	0.33	0.31	0.03	
Tree	0.949	0.28	0.26	0.22	0.46	0.25	0.26	0.14	0.50	0.947
$\delta^{15}\text{N}$	0.0001	0.22	0.27	0.35	0.04	0.30	0.27	0.56	0.03	0.0001

*p value entered below each corresponding coefficient.

N% = total N as a percentage of dry biomass.

$\delta^{15}\text{N}$ = per mill excess of ^{15}N isotope concentration as compared with atmosphere.

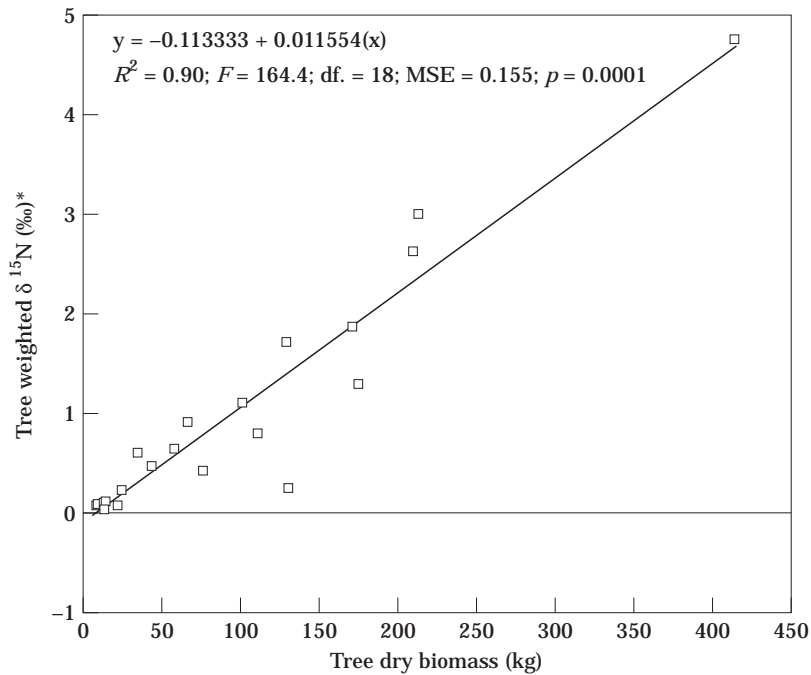


Figure 2. Relationship between tree dry biomass and tree weighted $\delta^{15}\text{N}$. (□) = observed values; (---) = regression line. * The sum of the weighted $\delta^{15}\text{N}$ of all tissues of the tree.

weighted average by standing biomass of $\delta^{15}\text{N}$ of the four tissue fractions added together. Figure 2 illustrates the positive relationship between tree dry biomass and $\delta^{15}\text{N}$. Trees in the 20–40 kg range had $\delta^{15}\text{N}$ values of about 0.3‰, whereas trees with a biomass of 400 kg had a $\delta^{15}\text{N}$ of approximately 5‰. This infers that the larger the tree the less the percentage of N in the tree is derived from N_2 fixation. Using Eqn (6) and different reference plants (Table 7), we calculated %Ndfa. Depending on the reference material this parameter ranged from 20% to 73% (Table 8).

Since the total tree biomass is also very highly correlated with the % N of the entire tree, this implies a strong demand for N. Given the 1200 kg more N under the canopy of these trees than outside the canopy (East & Felker, 1993), it would appear that the larger trees are relying on the increased soil N from prior periods. The corollary is that the smaller trees without access to this enhanced N pool resort to N_2 fixation to meet much of their N requirements. The translation of low $\delta^{15}\text{N}$ values for young trees and high $\delta^{15}\text{N}$ values for older trees to actual percentages of N derived from fixation (%Ndfa) is problematic due to several choices for reference plants for use in Eqn (6). In Shearer & Kohl's (1986) classic review of the use of natural ^{15}N abundance to estimate N_2 fixation, they stressed the need to use reference plants with the same rooting pattern as the 'test' plant so as 'to integrate the ^{15}N abundance of plant available soil N over time and depth'.

The $\delta^{15}\text{N}$ values of shrubs and grasses that we collected and values determined by Boutton *et al.* (1992) for the same species are provided in Table 7. Boutton's data were collected on the La Copita experimental site which is very similar to our site and is located only 30 km away. As the %Ndfa is linearly related to the difference in the $\delta^{15}\text{N}$ values of the reference plants (ranging from 3.37 to 5.89 in leaves and 1.34 to 6.18 in wood) there might appear to be a problem. However, Boutton's soil values have such low variability that it appears the low values for $\delta^{15}\text{N}$ of the reference plants arise either

Table 7. Tissue $\delta^{15}\text{N}$ in plants used as reference material

Reference species	$\delta^{15}\text{N}^*$ (‰)		
	leaves	wood	whole plant
Values obtained in this study[†]			
<i>Diospyros texana</i> (shrub)	5.89	6.18	–
<i>Celtis pallida</i> (shrub)	5.71	1.79	–
<i>Zanthoxylum fagara</i> (shrub)	3.37	1.34	–
<i>Setaria texana</i> (grass) (growing beneath mesquite)	–	–	4.71 (1.74) [‡]
<i>Setaria texana</i> (grass) (growing in the open)	–	–	7.83 (0.58) [§]
Values from literature at adjacent site (Boutton <i>et al.</i>, 1992)			
Non-fixing species ($N = 20$)	4.7 ± 1.2		
Total soil N ($N = 52$)	7.3 ± 0.6		

* $\delta^{15}\text{N}$ = per mill ^{15}N excess as compared to atmospheric ^{15}N .

[†] $\delta^{15}\text{N}$ values for *Diospyros* sp., *Celtis* sp. and *Zanthoxylum* sp. are from one composite sample from pooled leaves or twigs from all 20 plots from which the mesquite trees were harvested.

[‡] $\delta^{15}\text{N}$ mean and standard deviation of 20 observations.

[§] $\delta^{15}\text{N}$ mean and standard deviation of five observations.

from selective fractionation of fixed N_2 from other sources, i.e. *Prosopis*, or fixation by the reference plants from unknown endophytes.

Since the *Prosopis* at this site are non-phreatophytic and since non-phreatophytic *Prosopis* have dense extensive superficial feeder roots (Weltz & Blackburn, 1995), Kohl (1995, pers. comm.) suggested the need to examine the $\delta^{15}\text{N}$ of grasses grown under and away from *Prosopis* canopy. The objective of this determination would be to compare plants that would draw from the same N pool as superficial *Prosopis* roots. Thus we measured $\delta^{15}\text{N}$ of the grass *Setaria texana* Emery that is commonly associated with mesquite. The values for the grass growing under the canopy are significantly lower ($p = 0.0001$) than the values outside the canopy. Moreover, the values for grass growing outside the canopy are very similar to soil values as determined by Boutton *et*

Table 8. Effect of silviculture treatments on the percentage of N derived from N_2 fixation (%Ndfa*) in mesquite trees, as estimated using leaf $\delta^{15}\text{N}$ of different reference plants

	<i>Diospyros texana</i>	<i>Celtis pallida</i>	<i>Zanthoxylum fagara</i>	<i>Setaria</i> (beneath)	<i>Setaria</i> (open)
	% N biologically fixed				
C	69.4	64.7	48.2	59.4	72.7
T	57.4	50.6	27.9	44.8	62.9
UR+T	57.5	50.7	31.2	45.4	63.2
UR+T+H	52.7	45.2	20.0	47.6	64.8
UR+T+H+P	51.0	44.4	18.8	44.2	62.5

$$\% \text{Ndfa}^* = \frac{\delta^{15}\text{N reference} - \delta^{15}\text{N problem}}{\delta^{15}\text{N reference} - \delta^{15}\text{N N-free media}} \times 100$$

Note: In calculations of %Ndfa *Setaria* was used as the reference plant, and the tree weighted average of $\delta^{15}\text{N}$ ($\delta^{15}\text{N}$ of all tissues multiplied by the respective tissue total N in kg) was utilized.

al. (1992). The values for grass growing under the canopies apparently are depressed as a result of uptake of N recently derived from *Prosopis* N₂ fixation.

To avoid the problem of fractionation of $\delta^{15}\text{N}$ among various tissues within the plant, Shearer & Kohl (1986) suggested standardizing the differences by using the same tissue, i.e. leaves for both reference plants and the N₂ fixing species. However, since we have measured the $\delta^{15}\text{N}$ in each plant part and accurately know the relative biomass of each fraction, we have been able to arrive at a 'whole tree' $\delta^{15}\text{N}$ based on a biomass weighted average of the $\delta^{15}\text{N}$ values. It is legitimate to use this calculation in a comparison with the entire above-ground biomass of the grasses. It is interesting that the grasses collected beneath the trees have a similar value to the largest *Prosopis*, indicating little N₂ fixation by the large *Prosopis*. In contrast, the smallest *Prosopis* have much lower $\delta^{15}\text{N}$ values.

Thus we feel that the %Ndfa fixed by *Prosopis* will be bounded by the values obtained when: (1) *Prosopis* leaves are compared to leaves from the shrubs *Diospyros texana* Scheele, *Zantophyllum fagara* (L.) Sarg. and *Celtis pallida* Torr. and (2) when the weighted tree value of $\delta^{15}\text{N}$ is compared to the entire $\delta^{15}\text{N}$ of the grasses grown under and outside *Prosopis* canopies. The percentage of nitrogen derived from the atmosphere in mesquite was calculated using different reference plants (Table 7).

A comparison of the %Ndfa for each of the treatments, based on leaf and whole tree (for grasses) comparisons, is provided in Table 8. Given the fact that at the time of this study the larger trees were fixing less N₂ than the smaller trees, it seems reasonable that the treatments that allowed for tree development, i.e. thinning and P fertilization, would have the lowest %Ndfa. Thus the thinning treatment had less %Ndfa than the control treatment and the complete treatment containing P fertilization had the lowest %Ndfa regardless of the reference material used.

From the ranges in %Ndfa and the total gain in N over the 8 years it is possible to calculate the N₂ fixed over this period. Due to the fact that whole plant values of $\delta^{15}\text{N}$ for *Setaria texana* growing outside of the canopies closely matches the $\delta^{15}\text{N}$ obtained by Boutton *et al.* (1992) for soil outside the canopy, we believe that the %Ndfa calculated using this grass as reference plant was the best estimate. Using these estimates we calculated the N₂ fixation rate ranging from 15.9 kg N ha⁻¹ (8 year)⁻¹ for the treatment where P fertilizer had been applied, to 22.4 kg N ha⁻¹ (8 year)⁻¹ for the treatment where the understory vegetation was removed and the trees were thinned.

Discussion and conclusions

The silviculture and P fertilization treatments had little effect on the growth of the stand. Only the thinning treatment stimulated significant ($p = 0.0001$) enhanced percentage growth. Similarly, none of the silviculture treatments or P fertilization influenced N₂ fixation in the stand. Given the low soil P values of 2 mg kg⁻¹ Olsen available P under the canopies of the non-fertilized plots, the nearly 3-fold elevated soil P of 7.0 mg kg⁻¹ under the canopy of the fertilized trees (Muthaiya & Felker, 1996), and the strong influence of P fertilization on N₂ fixation in legumes, the lack of a P stimulation on N₂ fixation is perplexing.

Annual dry biomass growth was very low ranging from 460 to 700 kg ha⁻¹ year⁻¹ (0.65 to 1.0 m³ ha⁻¹ year⁻¹). In comparison, in non-irrigated plantations, the productivity of high biomass producing *Prosopis alba* clones and the mean of nine families of *P. glandulosa* var. *glandulosa*, only 35 km distant, was 13,000 kg ha⁻¹ year⁻¹ (Felker *et al.*, 1989) and 6900 kg ha⁻¹ year⁻¹ (Duff *et al.*, 1994), respectively.

It is unclear why this stand should have such low productivity. Perhaps it is competition from herbaceous cover, shrub cover or a lack of fertility. From a theoretical perspective, removal of the shrub understory and P fertilization would have appeared to have significantly improved the growth of the stand. The only major

difference between this native stand and the plantations 35 km distant was the elimination of herbaceous competition with herbicides and cultivation until stand closure.

There is the possibility that trace elements, i.e. copper, may have limited the growth of the stand. Minimal leaf copper levels determined in the greenhouse were 17 mg kg^{-1} for *Prosopis alba*, and 26 mg kg^{-1} for *P. glandulosa* (Cline *et al.*, 1986). Wightman & Felker (1990) found about 1 mg kg^{-1} leaf copper for *Prosopis alba* on a low productivity site. Muthaiya & Felker (1996) observed leaf copper levels on this site to be about 3 mg kg^{-1} which is considerably below the 26 mg kg^{-1} requirement determined in the greenhouse. In addition, Khan *et al.* (1996) determined that leaf Cu levels were significantly correlated to the productivity of another tree legume, *Leucaena leucocephala* (Lam.) de Wit in South Texas.

The absolute quantity of N_2 fixed in this study is considerably lower than the 35 kg ha^{-1} value reported by Rundel *et al.* (1982) and the values of 45 to 140 kg N ha^{-1} based on frequency of nodule occurrence reported by Johnson & Mayeux (1990). However the range in fractional contribution of fixed N_2 to total assimilated N determined in this study is comparable to the 43% to 61% reported for *Prosopis* across diverse sites in California by Shearer *et al.* (1983). Therefore the low N_2 fixation rates observed here are due to low productivity of the stand and not to a low %Ndfa.

If the high %Ndfa of up to 70% observed in young trees could be maintained with similar whole tree N concentrations, and the productivity observed of $6 \text{ Mg dry matter ha}^{-1}$ in plantations of *P. glandulosa* var. *glandulosa*, annual fixation rates of 26 kg ha^{-1} would appear to be possible.

The apparent problem with variation in $\delta^{15}\text{N}$ in fractions of tree and among shrubs and grasses should not be viewed as a problem but as a marvellous opportunity to examine N fluxes with a natural label in a complicated system. This is made possible by the high mean soil $\delta^{15}\text{N}$ value of $+7.3$ outside the canopy and the low standard deviation (0.5) that eliminates soil heterogeneity as responsible for differences in reference plants. Clearly reference plants with $\delta^{15}\text{N}$ values of 3–4 must be either: (1) tapping a soil source of more readily available N that was recently fixed by *Prosopis* (and thus lower $\delta^{15}\text{N}$); (2) the reference plant root must be discriminating various nutrients enriched in $\delta^{15}\text{N}$; (3) symbionts associated with reference plants are tapping recently fixed N_2 with lower $\delta^{15}\text{N}$; or (4) symbiont or the plant/symbiont couple is actively fixing N_2 . Whichever of these possibilities occurs, elucidation of the process by following the ^{15}N excess label should be very informative.

Stock *et al.* (1995) have experienced difficulty in assigning a percentage of fixed N_2 to *Acacia cyclops* G. Don and *A. saligna* (Labil.) Wendl. in South Africa due to low values for non-fixing reference plants on the site. Because the parent soils outside the influence of the fixing trees were higher in $\delta^{15}\text{N}$ than the non-fixing reference plants, we suggest the reference plants were tapping a source of N_2 recently fixed by *Acacia*. Careful fractionation of the soils associated with *Acacia* might lead to a $\delta^{15}\text{N}$ enriched fraction that was selectively available to the reference plants. In this situation, it would seem appropriate to compare the mean $\delta^{15}\text{N}$ of the entire *Acacia* plant (or weighted average of its parts) to the $\delta^{15}\text{N}$ value of an entire grass or shrub completely outside the influence of the *Acacia* plants.

Certainly in this case a comparison of the weighted average according to biomass of $\delta^{15}\text{N}$ per tree provided most useful results. This was true in the comparison of the weighted average to non-fixing plants and to the changes in nitrogen fixation with tree size and stand development.

The highly significant negative correlation ($r = -0.70$, $p = 0.001$, $N = 20$) between $\delta^{15}\text{N}$ and N concentration in the branches suggests that the branches are a first major sink of fixed N_2 from the roots. Seasonal sampling of cores from main trunk and branches, and harvest of leaves and twigs for $\delta^{15}\text{N}$ determinations should elucidate the translocation pattern of fixed N_2 in *Prosopis*.

Certainly the most significant result of this work is not the minor differences in %Ndfa between silviculture treatments, or the assessment of annual accretion of N₂ fixed by the stand, but the remarkable correlation between entire tree δ¹⁵N and biomass. The high percentage of N₂ fixed in young trees and the low percentage of N₂ fixed in older trees implies a transition, probably related to N accumulation in the soil, as the stand matures. Obviously, the identification of factors that influence this transition are of considerable significance both in ecological and economic terms.

While much is known about the factors controlling N₂ fixation at the cellular and whole plant level with annuals, there is little information on processes that control N₂ fixation at the stand level for N₂ fixing trees. Woody legumes of the genera *Prosopis* and *Acacia* occur on much of the third of the earth's surface that is semi-arid/arid (Griffith, 1961). The severe environmental degradation from grazing, firewood collection and desertification in these ecosystems clearly requires a firm basic understanding of the fluxes of N and C on which to base ecosystem recuperation. We hope that many additional studies will be undertaken to assist in our understanding of this critical world problem.

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