NITROGEN FIXATION IN FOUR DRYLAND TREE SPECIES IN CENTRAL CHILE

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Abstract

Results are presented from a 5-year experiment using ^{15}N-enriched fertilizer to determine N_2 fixation in four tree species on degraded soils in a Mediterranean-climate region of central Chile in which there are 5 months of drought. Species tested included three slow-growing but long-lived savannah trees native to southern South America, (*Acacia caven, Prosopis alba and P. chilensis*; *Mimosoideae*), and Tagasaste (*Chamaecytisus proliferus* ssp. *palmensis*; *Papilionoideae*), a fast-growing but medium-lived tree from the Canary Islands. Tagasaste produced four- to twenty-fold more biomass than the other species, but showed declining N_2 fixation and biomass accumulation during the 5th year, corresponding to the juvenile-to-adult developmental transition. Nitrogen content was significantly higher in Tagasaste and *Acacia caven* than in the other species. The data revealed inter-specific differences in resource allocation and phenology of N_2 fixation rarely detailed for woody plants in dryland regions.

1. INTRODUCTION

Although studies have been conducted on biological N_2 fixation (BNF) by trees of temperate and moist-tropical regions [1,2,3, other chapters in this volume], little is known of the factors that affect BNF by shrubs and trees in arid and semiarid areas [4,5]. Given the water deficits, the low levels of soil N and P, and variability in micro-symbiont rhizobial population density and composition, large variability in BNF and growth patterns in general is to be expected in N_2-fixing trees (NFTs). This variability needs to be better understood if NFTs are to be used for managing ecosystems more efficiently and effectively, and to aid the design of agroforestry systems [6-9].

An anthropogenic savanna-like formation, locally known as "espinales", occupies over 2 M ha of the Mediterranean climate region of central Chile [10]. Yields in this region are low, both in dryland farming systems and livestock husbandry. We have postulated that an invasive woody weed, also a NFT, has "parasitized" the espinales [10-12]. This species is *Acacia caven* (Mol.) Mol., the "espino" after which, along with several *Prosopis* species, the area is named. In the interior dry-lands of central Chile, where espinales dominate, approximately 300,000 people farm, graze and cut firewood intensively. These farmers generally lack the financial resources required to improve or even maintain agricultural productivity. Furthermore, there is increasing pressure on land-owners to sell their farmland for commercial purposes, such as paper-pulp production. Thus, there is an urgent need to improve these agroforestry systems with appropriate trees, pastures and crops [10,12-18].

We conducted a 5-year study with the objective of comparing BNF in three economically important *Mimosoideae* species from South America, and an unusually fast-growing *Papilionoideae* species from the Canary Islands, *Chamaecytisus proliferus*, that has potential for agroforestry systems in central Chile [17].

The data presented here cover a) annual and cumulative biomass in both above-and below-ground organs, and, for the NFTs, b) root nodulation and BNF, extending a report of the first 2 years of the experiment [14]. Trends apparent after 5 years' growth are discussed in terms of both ecological and applied aspects, including varying resource-uptake and -utilization strategies related to ontological strategy and biogeographic origin, as well as the niches these species could occupy in the development of agroforestry systems.

Genetic variability within species was not taken into account, even though such variation in BNF is known to occur [19]. Instead, a long-term in-situ approach was adopted in order to reveal year-to-year variations in the components studied.

2. MATERIALS AND METHODS

The experimental site was located in the sub-humid Mediterranean-climate zone of central Chile, in a field representative of local conditions [18] at the Instituto de Investigaciones Agropecuarias (INIA) Experimental Center in Cauquenes, in the sub-humid portion of the Mediterranean-climate zone of Chile (35°58'S; 72°17'W) with mean annual precipitation of 695 mm, and a summer drought period of 5-6 months. Soils are loamy and of granitic origin (Maule series in Chilean terminology). Prior to planting, six randomly spaced soil samples were analyzed, yielding the following baseline data for the 0-30 cm layer (mean ± SD): organic matter 1.45 ± 0.15%; pH 6.03 ± 0.35; total N 0.06 ± 0.0%; available N (ppm) 5.67 ± 4.82; available P (ppm) 2.5 ± 0.5; and available K (ppm) 95.8 ± 49.3. Total rainfall at the site for 1992 to 1996 was 975, 655, 502, 509 and 499 mm, respectively (Fig. 1).

2.1. Plant material

The following NFTs were evaluated: *Acacia caven, Prosopis alba, Prosopis chilensis* and *Chamaecytisus proliferus* ssp. *palmensis* ("Tagasaste"). The *Prosopis* species have a taller, more widely-spreading growth habit than the locally dominant *Acacia caven*, and thus potentially provide greater shade and canopy cover while bearing large quantities of palatable, nutritious pods [7,20]. Like *A. caven*, these *Prosopis* species are slow growing but long-lived desert or savanna trees of the *Mimosoideae* sub-family, and native to southern South America. Tagasaste, a fast-growing but short-lived *Papilionoideae* species tree endemic to La Palma, Canary Islands, is a valuable source of animal fodder in the Mediterranean-climate zones of Australia, Spain and elsewhere [21], and is well adapted to central Chile [14,22]. The two non-fixing reference species used for isotope-dilution calculations were the native *Schinus polygamus* (*Anacardiaceae*), and the introduced, but widely naturalized, *Fraxinus excelsior* (*Oleaceae*).

2.2. Rhizobial strains and nursery propagation

From preliminary examination (S. Dhillion, personal communication) it appears that Chilean espinal soils are particularly deficient in microbial biomass, including rhizobia. Therefore, inoculation of the legume trees was deemed advisable. Inoculum for the three native *Mimosoideae* was prepared from nodules collected in the field from tree roots, and then transported to the laboratory in Venoject tubes charged with a desiccant. Rhizobial strains were isolated on yeast-extract mannitol agar and conserved on YEM slants at 5°C. Separate strains for *Acacia caven* and the two *Prosopis* species were then multiplied in YEM broths incubated for 5-7 days at 25°C. A specific rhizobial strain for Tagasaste was obtained from the Phoenix Company, Tasmania. Fresh seeds of all species were scarified with rough sandpaper and pre-germinated (48 h) in an incubator at 20°C. Pre-germinated seeds of the NFTs were surface sterilized, and then inoculated with broth containing the appropriate rhizobial isolate for each species, immediately prior to planting in a shadehouse. Plastic sleeves (10×50 cm) were used, each containing 1.5 kg of a potting medium that favours root development. Seedlings were irrigated daily until well established, and held in the nursery for 7 months prior to field planting.

2.3. Experimental site and layout

The experiment had a randomized block design, with four replications per species, and included plots with and without ^{15}N that were established simultaneously. Labelled plots were used for non-destructive plant sampling for N_2-fixation measurements. Reference plants and N_2-fixing plants were planted together randomly with a square spacing of 2 m to minimize root-grafting between adjacent trees. The perimeter of each area amended with ^{15}N, and which contained four plants of each tree species, was trenched to a depth of 80 cm and lined with 0.8-mm plastic film.

Unlabelled plots were established to determine growth rates (aerial and below ground), nodule number and weight, and time courses of biomass accumulation, and N content of all plant parts through destructive, whole-plant sampling. The five plots were divided into four sub-plots comprising three plants of each NFT (or control). Each plot was used every year to provide sample plants.

Following planting, Type I plots were labelled by applying 18 kg N ha^{-1} as a solution of ammonium sulphate enriched in ^{15}N at 10 atom % excess. The soil was then cultivated with a walk-behind rototiller. The area of each labelled plot was 96 m^2 and thus 384 m^2 were labelled in total. Type II plots and the borders of Type I plots were fertilized with the same rate of unlabelled ammonium sulphate. In view of the known macro- and micro-nutrient deficiencies affecting the soil of the site, additional fertilizer was supplied in the planting holes of all trees: 100 g normal superphosphate, 15 g mixed K_2SO_4 and $MgSO_4$, and 0.5 g boron calcite.

The entire experimental site (0.4 ha) was fenced off to prevent damage by rabbits. The field was kept clear of weeds by herbicide application plus a twice-yearly hoeing around each tree.

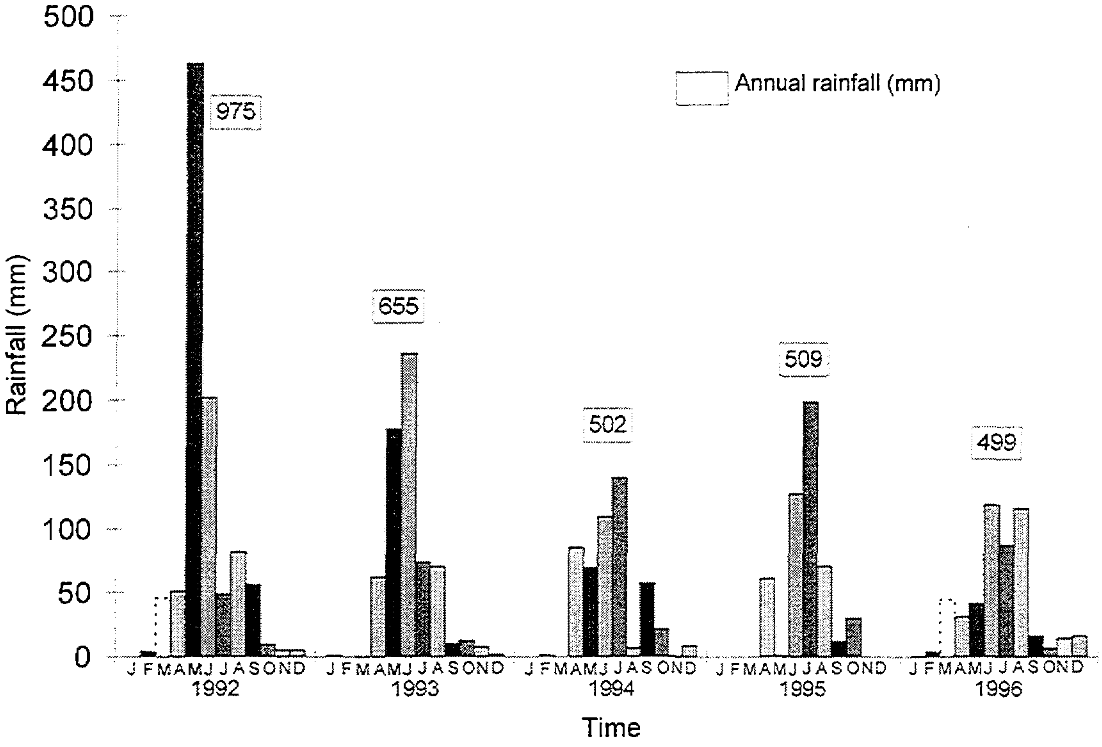

FIG. 1. Annual rainfall during the 5-year experimental period.

During the dry summer months of the first year, supplementary irrigation was applied each month at 15 L tree^{-1} to ensure good stand establishment. No further irrigation was applied.

2.4. Data collection and analysis

2.4.1. Type I plots

Growth rates were evaluated by measurement of tree height, trunk diameter and crown width following each season of active growth. Sampling for total N and isotope enrichment was carried out twice yearly: at the height of the most active growth (November) and at the end of the growing season (March). From each of the four trees on a plot, random samples of leaves were collected by hand and analyzed by mass-spectrometry at the FAO/IAEA Soil Science Unit, Seibersdorf, Austria.

The isotope-dilution method, for measuring the amount of N fixed, involves the application of a small amount of ^{15}N-enriched fertilizer to the soil. The NFT assimilates non-enriched atmospheric N_2 supplied by the root nodules [3, 23-25], in addition to the fertilizer-enriched mineral N in the soil. Accordingly, a comparison of the $^{15}N/^{14}N$ ratios of tissue N in the NFT and a non-fixing species allows an assessment of the relative contribution of BNF. The proportions and amounts of N derived from fixation (i.e. from the atmosphere, Ndfa) were obtained using the isotope-dilution equation [23]; average values for the two non-fixing control species are presented.

2.4.2. Type II plots

At the end of the active growing season of each year, three trees of each NFT were harvested from randomly selected sub-plots. Plant parts were separated into leaves, young twigs, lignified twigs and branches, nodules and roots, and then weighed following oven-drying at 60°C for 72 h. Root samples were divided into two groups: 0-40 cm and >40 cm. Statistical comparisons of mean values were made using Duncan's multiple-range test.

3. RESULTS

The data reveal trends and inter-specific differences in resource allocation and BNF rarely documented in similar detail for woody plants, whether in a Mediterranean-climate region, or elsewhere.

3.1. Plant growth

Plant-growth patterns in all six tree species are reported in Table I. Tagasaste grew faster than the other three NFTs and the two non-fixing reference species, as shown in height, trunk diameter, and root length. After 5 years, Tagasaste had attained an average height of 3.2 m, 8.6 cm in trunk diameter, and 2.1 m in rooting depth.

3.2. Biomass accumulation

The relatively poor performance of the two *Prosopis* species in terms of biomass production (Table II, Fig. 2) was probably due to poor adaptation to local conditions, in particular a sensitivity to the slightly acid soil pH. In contrast, total biomass accumulation by Tagasaste was consistently and significantly greater ($P<0.01$) than by the other NFTs – indeed, its total biomass production was nearly five times that of *A. caven*, and twenty times that of either Prosopis species. Plant height did not increase significantly in Tagasaste after the third year (Table I); instead, biomass was added in the form of a denser, and wider crown. The two reference species produced more than five-fold the biomass of either *Prosopis* species, slightly more than *A. caven*, but only one-quarter as much as Tagasaste (Table II).

TABLE I. GROWTH OF FOUR NFTs AND TWO REFERENCE SPECIES OVER 5 YEARS

Year	Plant height (m)					
	P. chilensis	*P. alba*	*A. caven*	*C. proliferus*	*F. excelsior*	*S. polygamus*
1992	0.91bc[a]	0.92bc	1.11b	1.87a	0.62d	0.88c
1993	1.36b	1.12b	1.38b	3.15a	1.12b	1.27b
1994	0.99c	1.08c	1.65b	3.21a	1.55b	1.56b
1995	1.02d	1.17d	1.71c	3.49a	2.20b	1.77c
1996	1.19d	1.43d	2.37b	3.21a	2.94a	1.90c
	Trunk diameter (cm)					
1992	1.40c	1.10d	1.60c	2.50a	2.20b	1.70c
1993	1.85d	1.86d	2.34c	5.44a	3.21b	2.97b
1994	2.65c	1.93d	3.04c	6.18a	3.82b	3.82b
1995	2.83d	2.60d	4.08c	9.18a	5.20b	5.19b
1996	3.20d	2.90d	4.90c	8.60a	6.60b	6.20b
	Root length (m)					
1992	1.39a	1.22ab	1.38a	1.20b	0.75c	0.82c
1993	1.62bc	1.56c	2.17a	1.89ab	1.55c	1.76bc
1994	1.82b	1.72b	2.31a	1.86b	1.74b	1.82b
1995	2.46a	1.99b	2.51a	2.39a	2.27ab	2.20ab
1996	1.93ab	1.79b	2.05a	2.08a	2.03a	1.93ab

[a] Numbers within rows followed by the same letter are not significantly different ($P<0.05$).

3.3. Nodulation

In each of the 5 years of growth, the weight of nodules on the roots of Tagasaste was at least four-fold that on any of the other NFTs (Table III). At the end of the fifth year (1996), Tagasaste's nodule weight averaged 113 g plant^{-1}, as compared to 2-5 g plant^{-1} on the other NFTs.

3.4. Nitrogen content of major plant components

The %N values for leaves, woody material and roots was significantly greater in the NFTs than in the two non-fixing species (Table IV). Moreover, Tagasaste showed significantly greater leaf-N concentration than did the other NFTs; its 2.75% N value for Tagasaste foliage appears to be exceptionally high, considering that leaf-N for other Mediterranean trees and shrubs is reported to be in the 1-1.5% range [26,27].

3.5. Total Nitrogen accumulation and %Ndfa

Tagasaste accumulated some five to thirty-fold more N per plant than did any of the other five species (Table V). Furthermore, both species of *Prosopis* accumulated significantly less total N than did the two reference species. By contrast, and despite its somewhat slower overall growth rate, *A. caven* consistently accumulated more N per plant than did the two reference species, and its %Ndfa was consistently high. The %Ndfa values for Tagasaste were also consistently high (ca. 80%) (Fig. 3). In contrast, following second-year peaks of 85%, 70%, and 52%, in *A. caven*, *P. chilensis* and *P. alba*, respectively, their %Ndfa values declined over the following 3 years.

3.6. Field scale estimates of BNF

To estimate fixed N on a per unit area basis (Table VI), we assumed a planting density of 1,666 trees ha^{-1} for all species, i.e. a spacing of 1.5 m between trees and 4 m between rows. This corresponds to accepted Tagasaste planting densities practised in areas with 650 mm of mean annual rainfall in both Chile and Australia. As reported previously [14], Tagasaste fixed approximately 8 and 74 kg N ha^{-1} in the first and second years, respectively, representing an order of magnitude greater than shown by the three mimosoid NFTs. In subsequent years, the gap only widened, with Tagasaste fixing a total of 367 kg ha^{-1}, some ten-fold higher than that fixed by *A. caven* and at least ninety-fold more than either of the *Prosopis* species.

TABLE II. BIOMASS ACCUMULATION IN FOUR NFTs AND TWO REFERENCE SPECIES OVER 5 YEARS

Year	*P. chilensis*	*P. alba*	*A. caven*	*C. proliferus*	*F. excelsior*	*S. polygamus*
	Aerial dry weight (g plant^{-1})					
1992	48b[a]	44b	80b	343a	42b	65b
1993	109b	162b	311b	4,141a	174b	341b
1994	228b	153b	637b	6,042a	349b	757b
1995	319c	336c	1,103b	10,251a	976c	1,240b
1996	340c	458c	2,205b	13,505a	2,210b	2,664b
	Root dry weight (g plant^{-1})					
1992	41c	35c	68c	108a	111b	43c
1993	107c	86c	203c	1,158a	417b	246c
1994	237c	149c	494bc	2,617a	858b	500bc
1995	320c	322c	665c	5,235a	1,587b	819c
1996	454c	398c	1,174bc	6,011a	2,493b	1,667bc
	Total-plant dry weight (g plant^{-1})					
1992	89b	78b	148b	451a	153b	108b
1993	216b	248b	514b	5,299a	591b	587b
1994	465b	302b	494bc	8,659a	1,207b	1,257b
1995	639c	657c	1,795b	15,486a	2,563b	2,059b
1996	794b	856b	3,379b	19,517a	4,703b	4,331b

[a] Numbers within rows followed by the same letter are not significantly different ($P<0.05$).

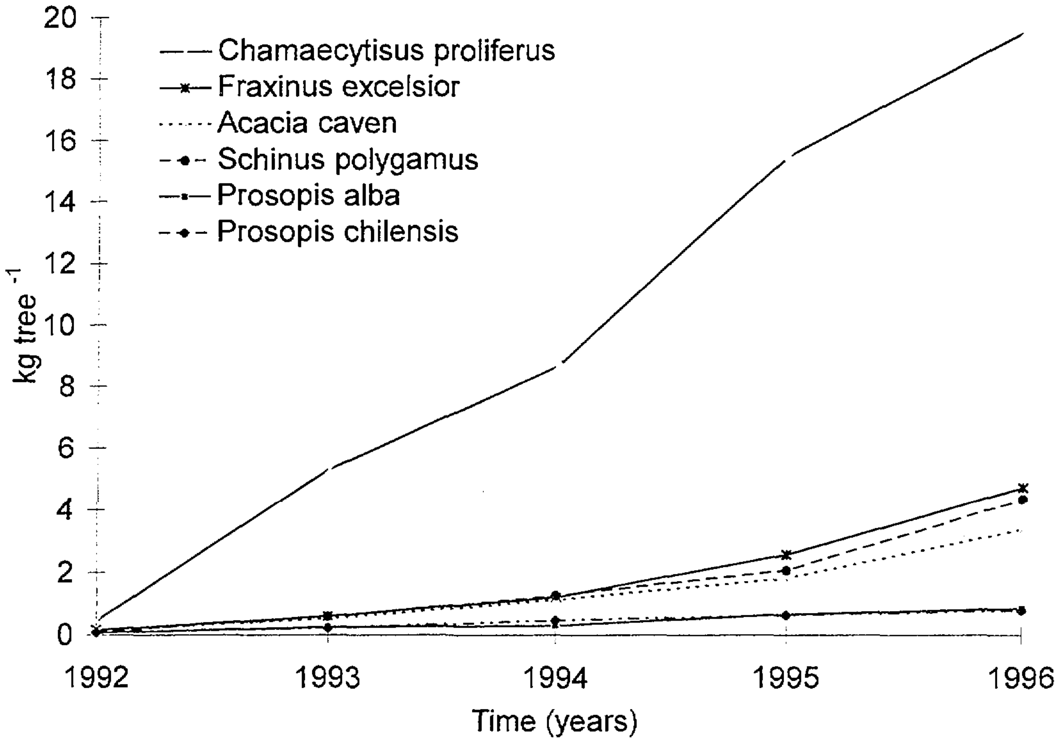

FIG 2. Total biomass accumulation in four legume and two reference trees over 5 years.

TABLE III. NODULE WEIGHT IN TWO SOIL DEPTHS ON FOUR NFTs OVER 5 YEARS

Year	0-40 cm (g plant^{-1})			
	P. chilensis	*P. alba*	*A. cavan*	*C. proliferus*
1993	1.5b[a]	3.6b	0.4b	18.5a
1994	0.38b	0.94b	0.36b	50.7a
1995	1.66b	3.41b	1.64b	59.3a
1996	3.28b	4.32b	2.01b	99.1a
	Deeper than 40 cm (g plant^{-1})			
1993	0.28b	0.35b	0.61b	9.11a
1994	0.15b	0.57b	0.47b	17.2a
1995	1.45b	2.74b	0.46b	12.5a
1996	0.08b	0.98b	0.12b	14.2a
	Total nodule dry weight (g plant^{-1})			
1992	1.2b	1.0b	1.5b	15.5a
1993	1.78b	3.95b	9.61b	18.6a
1994	0.53b	1.51b	0.83b	67.9a
1995	3.11b	6.15b	2.10b	71.8a
1996	3.36b	5.30b	2.13b	113a

[a] Numbers within rows followed by the same letter are not significantly different ($P<0.05$).

TABLE IV. NITROGEN CONCENTRATION IN THE COMPONENTS OF FOUR NFTs AND TWO REFERENCE SPECIES

Component	N concentration (%)					
	P. chilensis	*P. alba*	*A. caven*	*C. proliferus*	*F. excelsior*	*S. polygamus*
Leaf	1.71bc	1.93b	2.07	2.77a	1.38c	1.42c
Wood	1.04a	1.16a	1.03a	1.14a	0.72b	0.59b
Root	1.02b	1.09b	1.80a	1.92a	0.65c	0.54c

[a] Numbers within rows followed by the same letter are not significantly different ($P<0.05$).

TABLE V. CUMULATIVE TOTAL N AND BNF ESTIMATES FOR FOUR NFTs AND TWO REFERENCE SPECIES (n =12)

Year	Total N (g plant^{-1})					
	P. chilensis	*P. alba*	*A. caven*	*C. proliferus*	*F. excelsior*	*S. polygamus*
1992	0.97	0.95	2.22	5.53	1.27	0.83
1993	1.70	2.10	6.37	58.3	2.73	2.73
1994	3.46	2.56	13.7	91.5	5.31	5.90
1995	7.27	8.23	24.9	231	16.6	13.8
1996	8.38	9.61	49.2	270	38.6	35.3
	Fixed N (g plant^{-1})					
1992	0.30	0.24	0.31	4.81		
1993	1.19	1.10	5.45	49.1		
1994	0.96	0.48	10.1	76.2		
1995	1.15	0.64	12.8	192		
1996	2.18	1.35	23.7	220		

4. DISCUSSION

As compared to the other projects included in this Co-ordinated Research Project, the Chilean work is unusual in that it took place in a Mediterranean-climate region with 5 months of drought (Table I). Thus, the growth rates and BNF of the species tested were in general lower than those obtained in the other projects. Our relatively high year-to-year and intra-specific variability, in the destructive measurements of plants on the Type II plots, were in part due to genetic differences among individuals. This represents a source of error that can be eliminated only by using clonal material of selected tree species. However, a more critical step for the immediate future is to study these NFTs and others at the agro-ecosystem level. In other words, what happens to the N that is fixed by a given species in a research-site or on-farm study? For example, the fifth-year decline in

Tagasaste BNF was due apparently to the absence of biomass harvesting. Under normal conditions, this species would be directly grazed by livestock or pruned keeping it in a perpetual state of juvenility, therefore fixing N_2 at elevated rates. Tagasaste's significant production of N, ca. 73 kg ha^{-1} yr^{-1}, be managed in various ways, depending on the land-holder's objectives.

The next stage of our research will be aimed at developing optimum-use strategies for Tagasaste and other leading NFT candidates, in conjunction with annual legumes and grazing livestock. The isotope-dilution technique, and the stimulating interaction of an international network would certainly be of advantage for such endeavours.

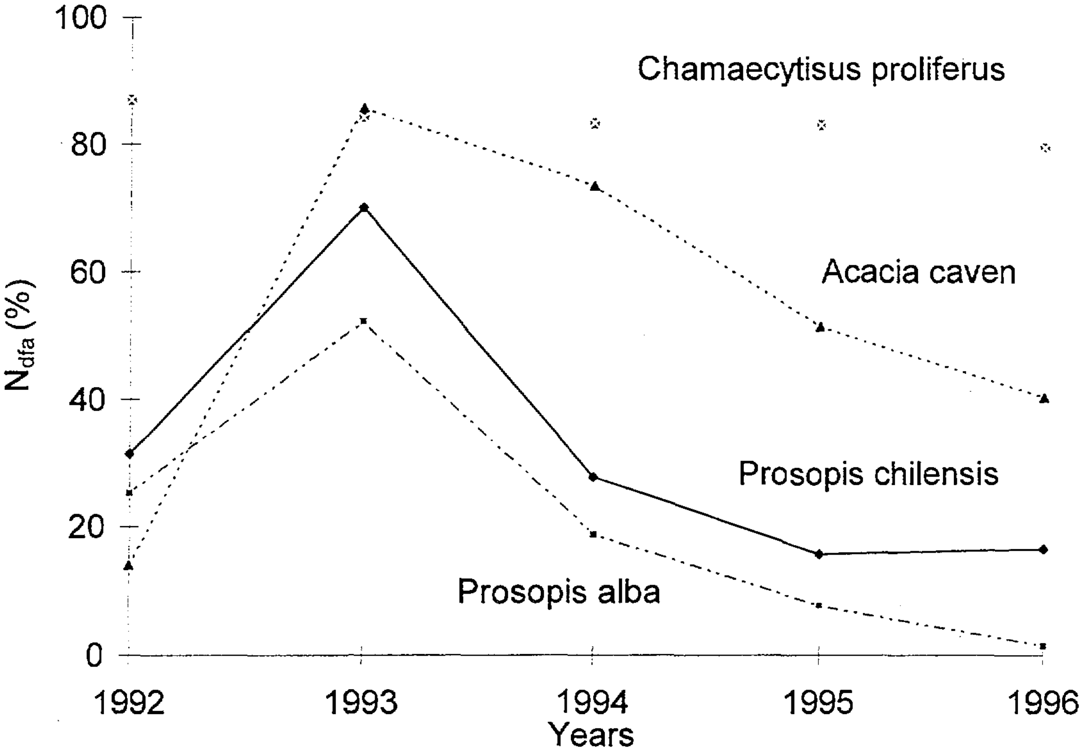

FIG. 3. %Ndfa in four NFTs over five years.

TABLE VI. ESTIMATES OF FIXED N FOR FOUR NFTs OVER 5 YEARS

Year	Fixed N (kg ha^{-1})			
	P. chilensis	*P. alba*	*A. caven*	*C. proliferus*
1992	0.50	0.40	0.52	8.01
1993	1.48	1.43	8.56	73.8
1994	0.00	0.00	7.70	45.1
1995	0.32	0.35	4.56	193
1996	1.72	1.19	18.1	46.5
Total	4.02	3.37	39.4	366

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REFERENCES

[1] MEAD, D.J., PRESTON, C.M., Nitrogen fixation in Sitka alder by ^{15}N isotope dilution after eight growing seasons in a lodgepole pine site, Can. J. For. Res. **22** (1992) 1192-1194.

[2] SANGINGA, N., et al., Nitrogen fixation of field-inoculated Leucaena leucocephala (Lam.) de Wit estimated using the ^{15}N and the difference methods, Plant Soil **117** (1989) 269-274.

[3] DANSO, S.K.A., et al., Biological nitrogen fixation in trees in agro-ecosystems, Plant Soil **141** (1992) 177-196.

[4] IZAGUIRRE-MAYORAL, M.L., et al., Quantitative analysis of the symbiotic N2-fixation, non-structural carbohydrates and chlorophyll content in sixteen native legume species collected in different savanna sites, Symbiosis **12** (1992) 293-312.

[5] NDOYE, I., et al., Nitrogen fixation in *Faidherbia albida*, *Acacia raddiana*, *Acacia senegal* and *Acacia seyal* estimated using the ^{15}N isotope dilution technique, Plant Soil **171** (1995) 175-180.

[6] NATIONAL RESEARCH COUNCIL. Tropical legumes: Resources for the Future, National Academy of Sciences, Washington, DC (1979).

[7] FELKER, P.F., Uses of tree legumes in semiarid regions, Econ. Bot. **35** (1981) 174-186.

[8] DOMMERGUES, Y., "The role of biological nitrogen fixation in agroforestry", Agroforestry. A Decade of Development (STEPPLER, H.A., NAIR, P.K.R., Eds.). ICRAF, Nairobi (1987) 245-271.

[9] ARONSON, J., et al., Restoration and rehabilitation of degraded ecosystems. I. A view from the South, Restoration Ecol. **1** (1993) 8-17.

[10] OVALLE, C., et al., The Espinal: agroforestry systems of the Mediterranean-type climate region of Chile: State of the art and prospects for improvement, Agrofor. Syst. **10** (1990) 213-239.

[11] ARONSON, J. Evolutionary biology of *Acacia caven* (*Leguminosae, Mimosoideae*). Intraspecific variation in fruits and seeds, Annals Missouri Bot. Gard. **79** (1992) 558-569.

[12] ARONSON, J., et al., "Land use changes and conflicts in central Chile", Landscape Disturbance and Biodiversity in Mediterranean-type Ecosystems (RUNDEL, P.W., et al., Eds.), Springer, Berlin (in press).

[13] DEL POZO, et al., Perspectivas de la rotación medicago-trigo en el secano interior de la zona Mediterránea, Agricultura Técnica (Chile) **49** (1989) 275-280.

[14] OVALLE, C., N_2 fixation, nodule efficiency and biomass accumulation after two years in Chilean legume trees and Tagasaste *Chamaecytisus proliferus* ssp. *Palmensis*, Plant Soil **179** (1996) 131-140.

[15] OVALLE, C., et al., Fixation d'azote par quatre légumineuses arborées en plantation agroforestière dans le Chili méditerranéen. L'agroforesterie pour un développement rural durable, Atelier International, Montpellier, France (1997) 357-360.

[16] ARONSON, J., et al., Restoration and rehabilitation of degraded ecosystems. II. Case studies in Chile, Tunisia and Cameroon, Restoration Ecol. **1** (1993) 68-187.

[17] OVALLE C., et al., Amélioration de l'espinal: système agro-sylvo-pastoral du Chili central, Options Médit. **12** (1995) 169-171.

[18] OVALLE C., et al., A study of the anthropogenic savannas in the subhumid region of central Chile. Land occupation patterns and vegetation structure. For. Ecol. Management **86** (1996) 129-139.

[19] SANGINGA, N., et al., Genetic variability in symbiotic nitrogen fixation within and between provenances of two Casuarina species using the ^{15}N-labelling methods, Soil Biol. Biochem. **22** (1990) 539-547.

[20] FELGER, R.S., "Ancient crops for the 21st century", New Agricultural Crops (RITCHIE, G.A., Ed.), AAAS Selected Symposium 38, Westview, Boulder, (1979) 5-20.

[21] SNOOK, L.C., Tagasaste (tree Lucerne) *Chamaecytisus palmensis*. A browse shrub which will increase production from grazing animals, Anim. Prod. Australia **15** (1989) 589-592.

[22] OVALLE, C., et al., Tagasaste (*Chamaecytisus proliferus* ssp. *palmensis*), un árbol forrajero leguminoso con potencial para sistemas agrosilvo-pastorales en Chile mediterráneo., Agric. Técnica (Chile) **53** (1993) 64-271.

[23] FRIED, M., MIDDELBOE, V., Measurement of the amount of nitrogen fixed by a legume crop, Plant Soil **47** (1977) 713-715.

[24] WEAVER, R.W., "Measurement of biological dinitrogen fixation in the field", Field Measurement of Dinitrogen Fixation and Dinitrification (Hauck, R.D., WEAVER, R.W., Eds.), Soil Science Society of America, Madison, (1986) 1-10

[25] PARROTTA, J.A., FRIED, M., Application of ^{15}N enrichment methodologies to estimate nitrogen fixation in *Casuarina equisetifolia*, Can. J. For. Res. **24** (1994 201-207.

[26] LEONARDI, S., RAPP, M., Retour au sol d'éléments minéraux et d'azote par l'intermédiaire des litières dans un écosystème à Quercus ilex L. du monte minardo (Etna), Flora **171** (1981) 329-337.

[27] ESCUDERO, A., et al., Materiales aportados al suelo por la encina en la zona de dehesas salmantina. II. Composición mineral. Studia, Oecologica **II** (1980) 213-240.