

BIOLOGICAL FIXATION OF N₂ IN MONO AND POLYSPECIFIC LEGUME PASTURE IN THE HUMID MEDITERRANEAN ZONE OF CHILE

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ABSTRACT

Despite annual legume pasture are of great importance for dryland agricultural systems in Mediterranean environments, there are few studies of N₂ biological fixation (NBF) reported in Chile. In this study the NBF of four annual legume species: subterranean clover (*Trifolium subterraneum* L.), yellow serradella (*Ornithopus compressus* L.), arrow-leaf clover (*T. vesiculosum* L.), and crimson clover (*T. incarnatum* L.) (Experiment 1), as well as seven mixtures of these species (Experiment 2) were assessed. The NBF was measured by the ¹⁵N natural abundance technique. The objective was to determine NBF in the legume species and in distinct mixtures used. The study was carried out in an Andisol of the Andean Precordillera located in the humid Mediterranean zone of Chile. Pasture was evaluated for biomass; and total N and natural abundance of ¹⁵N were analyzed in plant material samples. In Experiment 1 (monospecific legume species pasture), N derived from fixation ranged between 43 and 147 kg N ha⁻¹ and where *T. vesiculosum* and *T. subterraneum* presented statistical differences ($P \leq 0.05$) in connection with the other species. In the legume mixtures (Experiment 2), N derived by fixation varied between 97 and 214 kg N ha⁻¹ where the 50-50 mixtures (*T. subterraneum* and *O. compressus*, or *T. subterraneum* and *T. vesiculosum*, respectively) had the highest N fixation. Fixed N ranged between 12 and 25 kg N t⁻¹ DM, showing significant differences among mono and polyspecific legume species.

Key words: Natural abundance of ¹⁵N, Mediterranean pastures, volcanic soil.

INTRODUCTION

Legume pastures are the basis of pasture production in Mediterranean climate environments in the world as well as in Central Chile, and consequently, is the main source of nutrients for sheep and cattle production in these extensive agro-ecological areas. The two environmental factors mainly limiting pasture productivity in these environments are water availability associated to Mediterranean climatology (Loss and Siddique, 1994) and soil fertility conditions, particularly N and P supply (Lopes *et al.*, 2004). In this context, production systems based on legume fodder crops can play a fundamental

role in improving soil fertility, allowing efficient water and nutrient use, and weed control (Evans *et al.*, 2001). Thus, for farming systems based on permanent grazing of pastures where the establishment and maintenance fertilization cost is always higher, it is possible to achieve savings in N fertilizers through the selection of species and cultivars with a high N biological fixation (NBF) potential which is key to the success of a sustainable livestock activity.

Nitrogen biological fixation can directly contribute to agricultural production providing N from the vegetative parts of leaves, pods, seeds, and tubers of plants used as livestock feed or harvested for human consumption. NBF can also be an important source of N for agricultural soils through residues rich in N subsequent to plant harvest or grazing (Unkovich *et al.*, 2008). High value forage crops provide farmers with the capacity to diversify their production systems and are an integral part of strategies to intensify animal production (Unkovich *et al.*, 2008).

Legume species and cultivars differ in their N fixation capacity and in the biomass N content at the stem and root level and consequently, in the capacity to contribute N to the soil (Peoples *et al.*, 1995a; Urzúa, 2000; Fillery,

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2001; Campillo *et al.*, 2003; Ovalle *et al.*, 2006). The transfer of N from legumes to associated species in the pasture or to other crops in a legume-crop rotation system mainly occurs through the decomposition of their residues (Danso *et al.*, 1993; Peoples *et al.*, 1998).

Annual legumes mixtures increase plant diversity, productivity and pasture persistence (Tilman *et al.*, 1997; Avendaño *et al.*, 2005). A key aspect in the design of pasture mixtures is the correct selection of species and cultivars, which must combine different reproductive strategies and be able to establish and maintain an adequate seed bank in the soil for self-seeding after the crop face in a pasture-crop rotation (Loi *et al.*, 2000; Norman *et al.*, 2005; Ovalle *et al.*, 2005).

Isotopic methods applied to study N₂ fixation use two techniques, the isotopic dilution which use enriched fertilizer in ¹⁵N and the natural abundance of ¹⁵N (Boddey *et al.*, 1990). The former have been used to study the biochemistry of N₂ fixation and its quantification in legume crops, and factors affecting the N₂ fixation process, as well as to evaluate the efficiency of different microorganisms to fix N₂ and of the use of N fertilizer; the transfer of N in the soil or between plants and in studies of N balance. The natural abundance method has been used to evaluate NBF in natural ecosystems, because it does not involve excessive manipulation of the ecosystem, and also in legume pastures Mediterranean environments. This method has been proved to be reliable and provides accurate estimates of NBF in pastures and pulses (Ledgard and Steele, 1992; Doughton *et al.*, 1995; Peoples *et al.*, 1996; Unkovich *et al.*, 1997).

Very few studies of NBF have been reported for pastures in Chile (Herrera *et al.*, 1996; Campillo *et al.*, 2003; Ovalle *et al.*, 2006) and data of N fixation in Mediterranean pastures are particularly scarce. The objective of this study was to quantify NBF of four annual legume species and their respective mixtures using the ¹⁵N natural abundance technique in the humid Mediterranean in Chile.

MATERIALS AND METHODS

Site description and experiment management

The study was carried out in the Andean foothill of the Yungay county (37°10' S, 71°58' W, 297 m.a.s.l.), located in the humid Mediterranean zone of south-central Chile. The mean annual temperature is 14 °C, January being the warmest month and July the coldest, and with a 5-mo frost-free period (Novoa and Villaseca, 1989). Mean annual rainfall reaches 1200 mm (del Pozo and del Canto, 1999) and 4-mo (December to March) of dry season (Novoa and Villaseca, 1989). The soil at the site is an Andisol of loam texture, of the Santa Barbara soil

series (Typic Haploxerands; CIREN, 1999; Stolpe, 2006). Topography of the experimental site was slightly hilly. The soil presents levels of 5.8 pH, 15% OM, 4 mg kg⁻¹ soil inorganic N, 9 mg kg⁻¹ P, and 49 mg kg⁻¹ K at 20 cm depth. Previous crop was wheat (*Triticum aestivum* L.) and residues (equivalent to 2.5 t ha⁻¹) were incorporated to the soil in autumn.

Nitrogen fixation of four species of annual legumes (Experiment 1) and their respective mixtures (Experiment 2) were evaluated. In Experiment 1, the species were subterranean clover (*Trifolium subterraneum* cv. Mount Barker), yellow serradella (*Ornithopus compressus* cv. Ávila), arrow-leaf clover (*T. vesiculosum* cv. Zulu) and crimson clover (*T. incarnatum* cv. Corriente). In Experiment 2, seven mixtures of annual legume forage crops were evaluated; each mixture contained a proportion of 2, 3 or 4 species, and seed ratio was calculated based on the seed number to obtain 1000 plants per m² (Table 1). Reference plants were non-N fixing: annual ryegrass (*Lolium multiflorum* L. cv. Tama), orchard grass (*Dactylis glomerata* cv. Currie), tall fescue (*Festuca arundinacea* cv. Exella), and harding grass (*Phalaris aquatica* cv. Seed Master), established in the same year and experimental site. According to Boddey *et al.* (2000; 2001), a minimum of three reference species should considered due to variability in the quantity of N accumulated in biomass.

Pastures were sown in rows separated by 20 cm in 5 x 4 m plots, in both experiments, in a randomized complete block design with four replicates. Seeds were inoculated with the specific *Rhizobium* (10 g inoculant kg⁻¹ seed) for each species using methyl cellulose (1%) as a glue (1 L 25 kg⁻¹ seeds), and adding calcium carbonate (6-9 kg CaCO₃ 50 kg⁻¹ seeds) to cover and pellet the seeds. Fertilization in both experiments was the equivalent of 150 kg P₂O₅ ha⁻¹ (triple superphosphate), 500 kg CaSO₄ ha⁻¹ (calcium sulfate), 48 kg K₂O ha⁻¹ (potassium muriate), additionally 36 kg MgO, 44 kg K₂O and 44 S kg ha⁻¹ (sulpomag), and 2.2 kg B ha⁻¹ (boronatrocalcite), were applied before sowing (broadcast). No N fertilizer was applied.

Evaluations and determination of natural abundance of ¹⁵N

Above-ground biomass production was carried out by random collection in two 1 x 0.5 m quadrants, in each plot, in both experiments. Samples were oven-dried with forced air ventilation at 70 °C until a constant weight was reached for dry matter (DM) determination. Subsequently, a subsample of 1-2 g of plant material was sent to the Laboratory of Agrobiology EMBRAPA, Brazil, to determine total N (by Kjeldahl digestion) and the ¹⁵N natural abundance (δ¹⁵N) using a Finnigan Delta Plus continuous-flow isotope-ratio mass spectrometer

Table 1. Seed ratio (%) in the different annual legume mixtures and actual contribution (%) of the species in the mixture in November (in brackets) (Experiment 2).

Treatment (mixtures)	<i>Trifolium subterraneum</i> cv. Mount Barker	<i>Ornithopus compressus</i> cv. Ávila	<i>T. vesiculosum</i> cv. Zulu	<i>T. incarnatum</i> cv. Corriente
T1	50 (60)	50 (38)	-	-
T2	50 (8)	-	50 (92)	-
T3	50 (50)	-	-	50 (41)
T4	33 (14)	33 (25)	33 (60)	-
T5	33 (32)	-	33 (57)	33 (10)
T6	33 (32)	33 (48)	-	33 (13)
T7	25 (20)	25 (23)	25 (33)	25 (15)

Saving time in april.

interfaced with a Carlo Erba (Model EA 1108) automatic C-N analyzer (Finnigan- MAT, Bremen, Germany).

The ^{15}N natural abundance technique was used to estimate plant NBF. Three values of ^{15}N natural abundance were determined to estimate the proportion of N derived by biological fixation: a) the value β is obtained from inoculated legume plants with effective *Rhizobium* strains in an N-free medium which is then analyzed in terms of its $\delta^{15}\text{N}$ ratio (Peoples *et al.*, 1995a; 1995b) the abundance of ^{15}N -N derived from control plants, that is, non- N_2 fixing ($\delta^{15}\text{N}_{\text{ref}}$), and; c) the natural abundance of ^{15}N from N_2 -fixing plants ($\delta^{15}\text{N}_{\text{fix}}$). The percentage of N derived from air (%Ndfa), which is the proportional contribution of NBF to N in the legume, is calculated from the natural abundance of ^{15}N of the legume and the control plant as indicated in the following equation (Shearer and Köhl, 1986):

$$\% \text{Ndfa} = \frac{100 (\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{fix}})}{(\delta^{15}\text{N}_{\text{ref}} - \beta)}$$

The ^{15}N content in reference plants provides an integral estimate of available $\delta^{15}\text{N}$ in the soil during the whole growing period. Furthermore, it is assume that the pool of available ^{15}N in the soil is the same for both the reference plant and legumes (Boddey *et al.*, 2000).

In this study the value of β was -1‰ as have been

used by different authors in other fixation studies in Chile as well as in Australia (Unkovich *et al.*, 1994; Ovalle *et al.*, 2006). Most of the β values described for legume species oscillate between -2 and $+1\text{‰}$ (Köhl and Shearer, 1980; Shearer *et al.*, 1980; Steele *et al.*, 1983; Yoneyama *et al.*, 1986; Ledgard, 1989; Unkovich *et al.*, 1994; Boddey *et al.*, 2000). This variation is due legume-rhizobium association which can affect the natural abundance of ^{15}N in legumes (Köhl *et al.*, 1983; Steele *et al.*, 1983; Bergersen *et al.*, 1986; Yoneyama *et al.*, 1986).

All data were subjected to ANOVA ($P \leq 0.05$) previous test of normality. Mean separation was done by Duncan's multiple range test. All statistical analyses were carried out by SAS Systems for Windows V8 (SAS Institute, 1999).

RESULTS AND DISCUSSION

In Experiment 1, the highest production of DM was obtained in *O. compressus* cv. Ávila and *T. vesiculosum* cv. Zulu (9772 and 8830 kg ha^{-1}), significantly different ($P \leq 0.05$) from *T. subterraneum* cv. Mount Barker (6204 kg ha^{-1}), the latter being higher than the production of *T. incarnatum* cv. Corriente (3378 kg ha^{-1}) (Table 2). The percentage of N in the biomass was similar in all species but the accumulated N showed the same tendency as DM

Table 2. Dry matter production, N concentration, N accumulation, and natural abundance ($\delta^{15}\text{N}$) in monospecific pastures of annual legumes (Experiment 1).

Treatments (species)	Dry matter	N concentration	N accumulation	$\delta^{15}\text{N}$
	kg ha^{-1}	%	kg ha^{-1}	‰
<i>Trifolium subterraneum</i> cv. Mount Barker	6204b	3.60a	223b	-0.09b
<i>Ornithopus compressus</i> cv. Ávila	9772a	3.49a	341a	0.67a
<i>T. vesiculosum</i> cv. Zulu	8830a	4.10a	362a	0.42ab
<i>T. incarnatum</i> cv. Corriente	3378c	4.16a	141b	0.67a

Values with distinct letters in the columns are different according to Duncan multiple range test ($P \leq 0.05$).

(Table 2). The lowest $\delta^{15}\text{N}$ was found in *T. subterraneum*, which was significantly different ($P \leq 0.05$) from *O. compressus* and *T. incarnatum* (Table 2). This means that the natural abundance of ^{15}N was lower in *T. subterraneum* compared to *O. compressus* and *T. incarnatum*.

In Experiment 2, the higher biomass production was obtained in mixtures containing high proportion of *T. vesiculosum* at sowing and in the botanical composition (T2, T4 and T5), and in the mixture of *T. subterraneum* and *O. compressus* (T1), whereas the lower production was attained in mixtures with 25-50% of *T. incarnatum* at sowing (Table 3). The same tendency was observed in accumulated N, but N concentration was significantly different in the different mixtures (Table 3). The lowest $\delta^{15}\text{N}$ was obtained in the mixture of *T. subterraneum* and *O. compressus* (T1), indicating lower values of ^{15}N in the dry matter (Table 3).

Four gramineae species were used as reference plants to estimate NBF in both assays. The values of natural abundance of ^{15}N ($\delta^{15}\text{N}$) for each one were: annual ryegrass (1.26‰), orchard grass (1.31‰), tall fescue (1.39‰) and harding grass (1.59‰). In Experiment 1 (monospecific legumes), the highest %Ndfa was observed in *T. subterraneum*, however Ndfa (kg N

ha⁻¹) was similar ($P > 0.05$) to *O. compressus* and *T. vesiculosum*, due to differences in biomass production which masked differences in N fixed among species (Table 4). The N fixed, expressed as kg N t⁻¹ DM, fluctuated between 10 and 22, with statistical differences among *T. subterraneum* as compared to *O. compressus* and *T. incarnatum* (Table 4).

In Experiment 2, the %Ndfa was highest in the mixture of *T. subterraneum* and *O. compressus* (T1), which had the highest proportion of *T. subterraneum* in the biomass (Table 1 and 5). The Ndfa (kg N ha⁻¹) was higher in the mixture of *T. subterraneum* and *O. compressus* (T1), and the mixture with *T. vesiculosum* (T2, T3 and T4) (Table 5). The lower Ndfa was obtained in mixtures with 10-41% of *T. incarnatum* in the biomass (Table 1 and 5). Statistical differences ($P \leq 0.05$) were observed in the fixed N per unit of DM between the mixture of *T. subterraneum* and *O. compressus* (T1), and all the other mixtures (Table 5).

The results show the high potential of biomass production of legume pastures in volcanic soils of the Andean foothill which reached 11 t DM ha⁻¹ yr⁻¹ in some mixtures. These DM production are much higher than in other studies carried out in subhumid Mediterranean

Table 3. Aerial biomass production, N concentration, N accumulation, and ^{15}N natural abundance ($\delta^{15}\text{N}$) in specific annual legume mixtures (Experiment 2).

Treatments (mixtures)	Dry matter	N concentration	N accumulation	$\delta^{15}\text{N}$
	kg ha ⁻¹	%	kg ha ⁻¹	‰
T1	8 551abc ¹	3.77a	322abc	-0.19c
T2	11 386a	3.75a	427a	0.26b
T3	5 613c	3.87a	217c	0.33ab
T4	9 453ab	3.92a	371ab	0.43a
T5	9 379ab	3.81a	357abc	0.33ab
T6	6 993bc	3.71a	259bc	0.24b
T7	7 621bc	4.00a	305abc	0.50a

Values with distinct letters in the columns are different according to Duncan multiple range test ($P \leq 0.05$).

Table 4. Percentage of N derived from air in plants (% Ndfa) in the mixtures of four non-N fixing plants (control), N derived from air (Ndfa), and fixed N per unit of dry matter (Fixed N) (Experiment 1).

Treatments (species)	% Ndfa					Ndfa kg N ha ⁻¹	Fixed N kg t ⁻¹ DM
	Rye grass ¹	Orchard grass	Tall fescue	Harding grass	Mean		
<i>Trifolium subterraneum</i> cv. Mount Barker	60	61	62	65	62a	138a	22a
<i>Ornithopus compressus</i> cv. Ávila	26	28	30	35	30b	102ab	10b
<i>T. vesiculosum</i> cv. Zulu	37	39	41	45	41b	147a	17ab
<i>T. incarnatum</i> cv. Corriente	26	28	30	36	30b	43b	12b

¹Control plants.

Values with distinct letters in the columns are different according to Duncan multiple range test ($P \leq 0.05$).

Table 5. Percentage of N derived from air in plants (% Ndfa) in mixtures of four non-N fixing plants (control), N derived from air (Ndfa), and fixed N per unit of dry matter (Fixed N) (Experiment 2).

Treatments (mixtures)	% Ndfa				Mean	Ndfa kg N ha ⁻¹	Fixed N kg t ⁻¹ DM
	Rye grass ¹	Orchard grass	Tall fescue	Harding grass			
T1	64	65	66	69	66a	214a	25a
T2	44	45	47	51	47bc	202a	18b
T3	41	43	45	49	45bc	97b	18b
T4	37	38	40	45	40cd	148ab	16b
T5	41	42	44	49	44cd	158ab	17b
T6	45	47	48	52	48b	125b	18b
T7	33	35	37	42	37d	112b	15b

¹Control plants.

Values with distinct letters in the columns are different according to Duncan multiple range test ($P \leq 0.05$).

environments, in both Australia and Chile (Peoples *et al.*, 1995b; Dear *et al.*, 2004; del Pozo and Ovalle, 2009; Ovalle *et al.*, 2008; 2010). The lower water and nutrient availability in subhumid areas explained the lower productivity of pastures (Peoples *et al.*, 1995b; Unkovich *et al.*, 1997; Dear *et al.*, 2004), factors which are closely related to the fixed N quantities of the pastures.

Results of %Ndfa show important differences in the efficiency of the NBF among legumes species in mono and polyspecific pastures, in Andisols of the humid Mediterranean zone of Chile. In general, pastures with high proportion of *T. subterraneum* presented higher levels of %Ndfa. In soils with more limited fertility such as granites and Vertisols of the interior dryland of the subhumid zone, the Ndfa (%) values of *T. subterraneum* and *O. compressus* were 82-95% (Ovalle *et al.*, 2006). The high organic matter (16%) content and subsequent high mineralization capacity and N mineral availability in Andisols would explain the lower efficiency in the fixation process (Zagal *et al.*, 2003).

Despite the lower %Ndfa detected in some species, the amounts of fixed N per unit of area were high, especially in *T. vesiculosum* and in mixtures containing this species, as well as *T. subterraneum* and *O. compressus* (112 to 214 kg N ha⁻¹) (Table 5). This can be explained by the high biomass production of these pastures except the one containing 50% *T. incarnatum* (Table 3). These results agree with studies carried out in a perhumid environment in southern Chile, with similar N fixation values (190 kg N ha⁻¹) in *T. subterraneum* (Campillo *et al.*, 2003). In granitic soils and Vertisols of the subhumid Mediterranean zone (650 mm) the N fixation values were 41 and 56 kg N ha⁻¹ mainly as a consequence of the lower biomass productions (Ovalle *et al.*, 2006). In *Lotus corniculatus* L. in Vertisols under

irrigation and different cutting regimes, the amount of N fixed ranged from 112 to 173 kg N ha⁻¹yr⁻¹ (Ruz *et al.*, 1999).

In other Mediterranean environments in Australia, Peoples *et al.* (1995b) reported a wide range of N fixation from 2 to 206 kg N ha⁻¹ yr⁻¹ in pasture of *T. subterraneum*. Other studies carried out by Peoples *et al.* (1998) in northern Victoria and southern New South Wales indicate that efficiency was 20 to 25 kg N t⁻¹ DM produced by *T. subterraneum* pastures. Our results showed higher levels of N fixed per ton of DM in both monospecific pastures (10-22 kg N t⁻¹ DM, Experiment 1) and mixtures (15-25 kg N t⁻¹ DM, Experiment 2).

In summary, the amount of NBF by a legume pasture is depending of the species composition; however, other factors like the effectiveness of the *Rhizobium* strains, edaphoclimatic conditions, pasture management, and eventually, livestock management are also important. All these factors have an influence on $\delta^{15}\text{N}$ values but also in the DM production, which are the basis for estimating N fixation (Eriksen and Høgh-Jensen, 1998). In addition, soil N availability as well as small topographical variations provoking differences in water content or flooding can also influence NBF (Stevenson *et al.*, 1995).

CONCLUSIONS

From the studied annual forage species, *T. subterraneum*, *O. compressus* and *T. vesiculosum* fixed more N (average 129 kg N ha⁻¹) than *T. incarnatum* (43 kg N ha⁻¹). The higher NBF was reached in 50:50 legume mixtures of *T. subterraneum* and *O. compressus*, or *T. subterraneum* with *T. vesiculosum*, reaching an average of 208 kg N ha⁻¹, demonstrating synergy between these species in Chilean Mediterranean humid zone.

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RESUMEN

Fijación biológica de N₂ en praderas mono y poliespecíficas de leguminosas en la zona mediterránea húmeda de Chile. A pesar de la gran importancia que las praderas de leguminosas tienen en los sistemas agrícolas de secano en ambientes mediterráneos, existe muy poca información sobre la fijación biológica de N₂ (FBN) reportada en Chile. En este estudio se evaluó la FBN en cuatro leguminosas forrajeras anuales: trébol subterráneo (*Trifolium subterraneum* L.), serradela amarilla (*Ornithopus compressus* L.), trébol vesiculoso (*T. vesiculosum* L.) y trébol encarnado (*T. incarnatum* L.) (Experimento 1), además de siete mezclas de estas especies (Experimento 2). La FBN se midió mediante la técnica de la abundancia natural de ¹⁵N. El objetivo fue determinar la FBN en las especies de leguminosas y en las diferentes mezclas. El estudio se llevó a cabo en un suelo Andisol, de la Precordillera Andina, localizada en la zona mediterránea húmeda de Chile. En la pradera se evaluó producción de biomasa y en submuestras se analizó N total y abundancia natural de ¹⁵N. En el Experimento 1, el N derivado de la fijación fluctuó entre 43 y 147 kg N ha⁻¹; siendo *T. vesiculosum* y *T. subterraneum* las que presentaron diferencias estadísticas ($P \leq 0,05$) con respecto a las otras especies en estudio. En las mezclas de leguminosas (Experimento 2) el N derivado de la fijación fluctuó entre 97 y 214 kg N ha⁻¹; siendo las mezclas 50-50 (*T. subterraneum* - *O. compressus* y *T. subterraneum* - *T. vesiculosum*, respectivamente) las que presentaron la mayor fijación de N por hectárea. El N fijado fluctuó entre 12 y 25 kg N t⁻¹ MS, presentando diferencias significativas entre especies de leguminosas mono y poliespecíficas.

Palabras clave: abundancia natural de ¹⁵N, praderas mediterráneas, suelo volcánico.

LITERATURE CITED

- Avendaño, J., C. Ovalle, A. del Pozo, F. Fernández, and C. Porqueddu. 2005. Mezclas de trébol subterráneo con otras leguminosas anuales para suelos graníticos del secano mediterráneo subhúmedo de Chile. *Agricultura Técnica* 65:165-176.
- Bergersen, F.J., G.L. Turner, N. Amarger, F. Mariotti, and A. Mariotti. 1986. Strain of *Rhizobium lupini* determines natural abundance of ¹⁵N in root nodules of *Lupinus* spp. *Soil Biology and Biochemistry* 18:97-101.
- Boddey, R.M., M.B. Peoples, B. Palmer, and P.J. Dart. 2000. Use of the ¹⁵N natural abundance technique to quantify biological nitrogen fixation by woody perennials. *Nutrient Cycling in Agroecosystems* 57:235-270.
- Boddey, R.M., J.C. Polidoro, A.S. Resende, B.J.R. Alves, and S. Urquiaga. 2001. Use of the ¹⁵N natural abundance technique for the quantification of the contribution of N₂ fixation to sugar cane and other grasses. *Australian Journal of Plant Physiology* 28:889-895.
- Boddey, R.M., S. Urquiaga, M.C.P. Neves, A.R. Suhet, and J.R. Peres. 1990. Quantification of contribution of N₂ fixation to field-grown grain legumes. A strategy for the practical application of the ¹⁵N isotope dilution technique. *Soil Biology and Biochemistry* 22:649-655.
- Campillo, R., S. Urquiaga, I. Pino, and A. Montenegro. 2003. Estimación de la fijación biológica de nitrógeno en leguminosas forrajeras mediante la metodología del ¹⁵N. *Agricultura Técnica* 63:169-179.
- CIREN. 1999. Descripciones de suelos, materiales y símbolos. Estudio agrológico VIII Región. Vol. II. p. 289-586. Centro de Información de Recursos Naturales (CIREN), Santiago, Chile.
- Danso, S.K.A., G. Hardarson, and F. Zapata. 1993. Misconceptions and practical problems in the use of ¹⁵N soil enrichment techniques for estimating N₂ fixation. *Plant and Soil* 152:25-52.
- Dear, B.S., M.B. Sandral, B.C. Peoples, J.N. Wilson, and C.A. Rodham. 2004. Growth, seed set and nitrogen fixation of 28 annual legume species on three Vertisol soils in southern New South Wales. *Australian Journal of Agricultural Research* 43:1101-1115.
- del Pozo, A., and P. del Canto. 1999. Áreas agroclimáticas y sistemas productivos en la VII y VIII regiones. Serie Quilamapu N° 113. 115 p. Instituto de Investigaciones Agropecuarias, Chillán, Chile.
- del Pozo, A., and C. Ovalle. 2009. Productivity and persistence of serradela (*Ornithopus compressus*) and biserrula (*Biserrula pelecinus*) in the Mediterranean climate region of central Chile. *Chilean Journal of Agricultural Research* 69:340-349.
- Doughton, J.A., P.G. Saffigna, I. Vallis, and R.J. Mayer. 1995. Nitrogen fixation in chickpea. II. Comparison of ¹⁵N enrichment and ¹⁵N natural abundance methods for estimating nitrogen fixation. *Australian Journal of Agricultural Research* 46:225-236.

- Eriksen, J., and H. Hogh-Jensen. 1998. Variations in the natural abundance of ^{15}N in ryegrass/white clover shoot material as influenced by cattle grazing. *Plant Physiology* 68:48-52.
- Evans, J., A.M. McNeill, M.J. Unkovich, N.A. Fettell, and D.P. Heenan. 2001. Net nitrogen balances for cool-season grain legume crops and contributions to wheat nitrogen uptake: a review. *Australian Journal of Experimental Agriculture* 41:347-359.
- Fillery, I. 2001. The fate of biological fixed nitrogen in legume-based dryland farming systems. *Australian Journal of Experimental Agriculture* 41:361-381.
- Herrera, A., L. Longeri, and C. Ovalle. 1996. Estudio de la efectividad de cepas chilenas de *Rhizobium meliloti* en simbiosis con *Medicago polymorpha*. *Agricultura Técnica* 56:36-42.
- Köhl, D.H., B.A. Bryan, and G. Shearer. 1983. Relationship between N_2 -fixing efficiency and natural ^{15}N enrichment of soybean nodules. *Plant Physiology* 73:514-516.
- Köhl, D.H., and G. Shearer. 1980. Isotopic fractionation associated with symbiotic N_2 fixation and NO_3^- uptake by plants. *Plant Physiology* 66:51-56.
- Ledgard, S.F. 1989. Nutrition, moisture and rhizobial strain influence isotopic fractionation during N_2 fixation in pasture legumes. *Soil Biology and Biochemistry* 21:65-68.
- Ledgard, S.F., and K.W. Steele. 1992. Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil* 141:137-53.
- Loi, A., B. Nutt, R. McRobb, and M. Erwing. 2000. Potential new alternative annual pasture legumes for Australian Mediterranean farming system. *Cahiers Options Méditerranéennes* 45:50-54.
- Lopes, M., S. Nogués, and J.L. Arous. 2004. Nitrogen source and water regime effects on barley photosynthesis and isotope signature. *Functional Plant Biology* 31:995-1003.
- Loss, S.P., and K.H.K. Siddique. 1994. Morphophysiological and physiological traits associated with wheat yield increases in Mediterranean environments. *Advances in Agronomy* 52:229-275.
- Norman, H., P. Cocks, and N. Galwey. 2005. Annual clovers (*Trifolium* spp.) have different reproductive strategies to achieve persistence in Mediterranean-type climates. *Australian Journal of Agricultural Research* 56:33-43.
- Novoa, R., and S. Villaseca. 1989. Mapa agroclimático de Chile. 221 p. Instituto de Investigaciones Agropecuarias, Santiago, Chile.
- Ovalle, C., S. Arredondo, A. del Pozo, F. Fernández, J. Chavarría, and A. Augusto. 2010. Arrowleaf clover (*Trifolium vesiculosum* Savi): a new species of annual legumes for high rainfall areas of the Mediterranean climate zone of Chile. *Chilean Journal of Agricultural Research* 70:170-177.
- Ovalle, C., J. Avendaño, A. del Pozo, E. Zagal, S. Urquiaga, and J. Aronson. 2008. The effect of trees and pastures on the N contribution and productivity of an agroforestry system in Mediterranean central Chile. *In* Management of agroforestry systems for enhancing resource use efficiency and crop productivity. IAEA TEC DOC-1606:157-166.
- Ovalle, C., A. del Pozo, S. Arredondo, and J. Chavarría. 2005. Crecimiento y producción de nuevas leguminosas forrajeras anuales en la zona mediterránea de Chile. I. Comportamiento de las especies en la precordillera andina. *Agricultura Técnica* 65:35-47.
- Ovalle, C., S. Urquiaga, A. del Pozo, E. Zagal, and S. Arredondo. 2006. Nitrogen fixation in six forage legumes in Mediterranean central Chile. *Acta Agriculturae Scandinavica Section B- Soil and Plant Science* 56:277-283.
- Peoples, M.B., R.R. Gault, G.J. Scammell, B.S. Dear, J. Virgona, G.A. Sandral, *et al.* 1998. Effect of pasture management on the contributions of fixed N to the N economy of ley-farming Systems. *Australian Journal of Agricultural Research* 49:459-474.
- Peoples, M.B., D.F. Herridge, and J. Ladha. 1995a. Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant and Soil* 174:3-28.
- Peoples, M.B., D.M. Lilley, V.F. Burnett, A.M. Ridley, and D.L. Garden. 1995b. Effects of surface application of lime and superphosphate to acid soils on growth and N_2 fixation by subterranean clover in mixed pasture swards. *Soil Biology and Biochemistry* 27:663-671.
- Peoples, M.B., B. Palmer, D.M. Lilley, L.M. Duc, and D.F. Herridge. 1996. Application of ^{15}N and xylem ureide methods for assessing N_2 fixation of three shrub legumes periodically pruned for forage. *Plant and Soil* 182:125-37.
- Ruz, E., E. Acuña, E. Zagal, L. Barrientos, y A. Pincheira. 1999. Variación en las tasas de fijación de nitrógeno en tres especies del género *Lotus* por efecto del corte y del pastoreo. *Agricultura Técnica* 59:35-44.
- SAS Institute. 1999. SAS Release 8.1 Edition. SAS Institute, Cary, North Carolina, USA.
- Shearer, G., and D.H. Köhl. 1986. N_2 -fixation in field settings: estimations based on natural ^{15}N abundance. *Australian Journal of Plant Physiology* 13:699-756.

- Shearer, G., D.H. Köhl, and J.E. Harper. 1980. Distribution of ¹⁵N among plant parts of nodulating and non-nodulating isolines of soybeans. *Plant Physiology* 66:57-60.
- Steele, K.W., P.M. Bonish, R.M. Daniel, and G.W.O. Hara. 1983. Effect of rhizobial strain and host plant on nitrogen isotopic fractionation in legumes. *Plant Physiology* 72:1001-1004.
- Stevenson, F., J. Knight, and C. van Kessel. 1995. Dinitrogen fixation in pea: controls at the landscape and micro-scale. *Soil Science Society of America Journal* 59:1603-1611.
- Stolpe, N.B. 2006. Descripciónes de los principales suelos de la VIII Región de Chile. 112 p. Universidad de Concepción, Departamento de Suelos y Recursos Naturales, Chillán, Chile.
- Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie, and E. Siemann. 1997. The influence of functional diversity and composition on ecosystem processes. *Science* 277:1300-1302.
- Unkovich, M., D. Herridge, M. Peoples, G. Cadisch, B. Boddey, K. Giller, B. Alves, and P. Chalk. 2008. Measuring plant-associated nitrogen fixation in agricultural systems. *ACIAR Monograph* N° 136. 258 p.
- Unkovich, M.J., J.S. Pate, and P. Sanford. 1997. Nitrogen fixation by annual legumes in Australian Mediterranean agriculture. *Australian Journal of Agricultural Research* 48:267-293.
- Unkovich, M.J., J.S. Pate, P. Sanford, and R.L. Armstrong. 1994. Potential precision of the δ¹⁵N natural abundance method in field estimates of nitrogen fixation by crop and pasture legumes in S.W. Australia. *Australian Journal of Agricultural Research* 45:119-132.
- Urzúa, H. 2000. Fijación simbiótica de nitrógeno en Chile: Importante herramienta para una agricultura sustentable. p. 211-227. Proc. XX Reunión Latinoamericana de Rhizobiología, Arequipa, Perú.
- Yoneyama, T., K. Fujita, T. Yoshida, T. Matsumoto, and I. Kambayashi. 1986. Variation in natural abundance of ¹⁵N among plant parts and in ¹⁵N/¹⁴N fractionation during N₂ fixation in the legume rhizobia symbiotic system. *Plant and Cell Physiology* 27:791-799.
- Zagal, E., N. Rodríguez, I. Vidal, and G. Hofmann. 2003. Eficiencia de uso y dinámica del nitrógeno en una rotación con y sin uso de residuos. *Agricultura Técnica* 63:298-310.