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Short-term nitrogen fixation by legume seedlings and resprouts after fire in Mediterranean old-fields

P. CASALS[1,4,*], J. ROMANYA[2] and V.R. VALLEJO[3,4]
*[1]Area d'Ecologia Vegetal i Botànica Forestal, Centre Tecnològic Forestal de Catalunya, Pujada del Seminari s/n, E 25280 – Solsona, Spain; [2]Departament Productes Naturals, Biologia Vegetal i Edafologia, Universitat de Barcelona, Avgda, Joan XXIII s/n, E 08028-Barcelona, Spain; [3]Fundación Centro de Estudios Ambientales del Mediterráneo (CEAM), Parque Tecnológico c/ Ch, Darwin, 14, E-46980 Paterna, Spain; [4]Departament Biologia Vegetal, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, E 08028-Barcelona, Spain; *Author for correspondence (e-mail: pere.casals@ctfc.es; fax: +34-93-4112842)*

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Abstract. Fires may greatly alter the N budget of a plant community. During fire nitrogen is lost to the atmosphere. Although high light availability after fire promotes N_2-fixation, the presumably high soil N availability could limit N_2-fixation activity. The latter limitation might be particularly acute in legume seedlings compared with resprouts, which have immediate access to belowground stored carbon. We wished to learn whether early post-fire conditions were conducive to N_2-fixation in leguminous seedlings and resprouts in two types of grassland and in a shrubland and whether seedlings and resprouts incurred different amounts of N_2-fixation after fire. We set 18 experimental fires in early autumn on 6 plots, subsequently labelling 6 subplots (2×2 m^2) in each community with $^{15}NH_4^+$-N (99 atom % excess). For 9 post-fire months we measured net N mineralisation in the top 5 cm of soil and we calculated the fraction of legume N derived from the atmosphere (%Ndfa) in seedlings and resprouts. We used two independent estimates of the amounts of N derived from non-atmospheric sources in potentially N_2-fixing plants: mean soil pool abundance and the ^{15}N-enrichment of non-legumes. Despite substantial soil net N mineralisation in all burned community types (about 2.6 g Nm^{-2} during the first nine months after fire), the %Ndfa of various legume species was 52–99%. Legumes from both grasslands showed slightly higher N_2-fixation values than shrubland legumes. As grassland legumes grew in more belowground dense communities than shrubland legumes, we suggest that higher competition for soil resources in well established grass-resprouting communities may enhance the rate of N_2-fixation after fire. In contrast to our hypothesis, legume seedlings and resprouts from the three plant communities studied, had similar %Ndfa and apparently acquired most of their N from the atmosphere rather than from the soil.

Introduction

In temperate regions, legumes are abundant in the early stages of succession (Crews 1999) and have been identified as an important component of post-fire successional communities in Mediterranean climate regions (e.g. Naveh 1967; Bell and Kock 1980). The N losses caused by wildfires may be partially offset by the introduction of fixed atmospheric N_2 (e.g. Hanes 1971; Rundel 1981; Hendricks and Boring 1992). However, the extent to which legumes replace N lost to burning is not widely documented, and this is partially due to the lack of an accurate technique for quantifying N_2-fixation.

The dominance of legumes in post-fire communities has been attributed to their ability to fix atmospheric N_2 (e.g. Rundel 1981; Arianoutsou and Thanos 1996). However, legumes can also use the high levels mineralised, post-fire soil N (Vitousek and Howard 1991; Mckey 1994). Legumes in burned areas persist in early post-fire successional stages. As succession progresses, legumes are reduced in species numbers and area of plant cover (Arianoutsou and Thanos 1996). Rastetter et al. (2001) suggested that this gradual reduction is caused by the optimisation of resource allocation in the vegetation which limits N_2-fixation to periods when it is less costly than soil N uptake. According to Rastetter et al.'s (2001) Multiple Element Limitation model, legume N_2-fixation is more economical than soil N uptake when the canopy is open, the soil is well exploited by roots, soil inorganic N concentrations are low and other soil resources (P, water) are readily available. Modelling post-fire N_2-fixation, Vitousek and Field (1999) described a pulse of growth and activity of fixers following fire; they related this pulse to the temporary removal of shade and the enhancement of P availability. Because it is known that high levels of mineral N can depress nodulation and N_2-fixation (Waterer and Vessey 1993; Marschner 1995), Vitousek and Field (1999) suggested that as a consequence of high soil N availability their model probably overestimated post-fire N_2-fixation.

Because of land abandonment and fire, early successional grasslands and scrublands have become the dominant vegetation in the eastern Iberian Peninsula (Masalles and Vigo 1987). In those fire-prone ecosystems, rapid post-fire plant regeneration occurs via two main strategies: proliferation of seedlings from the soil seed bank and resprouting from the belowground vegetative parts of some plants (Naveh 1975; Keeley and Zedler 1978; Spetch 1981). Seedlings and resprouts have different structural and physiological responses to fire (Pate et al. 1990; Bell and Ojeda 1999); legume seedlings and resprouts may differ in their post-fire amounts of dependence on fixing atmosphere N_2-fixation. Studying a Mediterranean Basin ecosystem, Papavassiliou and Arianoutsou (1993) found potentially N_2-fixing nodules on legume seedlings, however, their N_2-fixation remains untested (Arianoutsou and Thanos 1996). The temporary post-fire increase of soil mineral N may limit nodulation capacity and their N_2-fixation activity; this may be especially important in legume seedlings because their shallow, young root systems mainly lie in the soil volume of intense soil N mineralisation. In contrast, the mostly unaffected belowground parts of resprouting legumes may be ready to fix atmospheric N_2 immediately after fire. In this way, Rastetter et al. (2001) and Vitousek et al. (2002) considered that a soil which is well exploited by the fine roots of leguminous resprouters favours N_2-fixation.

In this study we aim to know if, according to Vitousek and Field (1999) simulations, early post-fire conditions are conducive for N_2-fixation, and we hypothesized that, in such conditions, N_2-fixation by seedling legumes will be hindered to a greater extend than N_2-fixation by legume resprouts. The objectives of this study were to know, for three common Mediterranean plant communities: (1) the extent to which recovering legumes depended on N_2-fixation after fire, and (2) whether seedling and resprouting legumes accumulated fixed atmospheric N_2 in different amounts.

We used $^{15}NH_4^+$ to label the soil N-pool produced immediately after the fire and calculated the N_2-fixation of seedling and resprouting legumes regenerating during the first nine months. We assessed the N_2-fixation by the enriched ^{15}N isotopic dilution method using two independent estimates of the amounts of N derived from non-atmospheric sources in potentially N_2-fixing plants, the mean soil pool abundance technique (Barraclough 1991) and ^{15}N-enrichment of non-legumes.

Material and methods

Study area

The study was done in a set of abandoned fields in the northeastern Iberian Peninsula (41°56′ N, 0°37′ E, 460 m a.s.l.). The climate is continental Mediterranean, mean annual precipitation is 535 mm, and mean annual temperature is 12.4 °C. Terraced and cultivated until the early 1960s, the soils are Calcaric Cambisol (FAO, 1990) developed from a fine textured Eocenic limestone and marl colluvium with a pH (H_2O) of 8.2 and containing 40% of calcium carbonate. Organic C concentration in the top 5 cm of soil is about 29.7 g kg^{-1} and total nitrogen is 3.9 g kg^{-1}. Bulk density in the top 5 cm ranged from 0.8 to 1.2 g cm^{-3} depending on stoniness. After agricultural abandonment, the terraces were colonized by a mosaic of plant communities. Three types of plant communities, which are widely distributed in the Mediterranean basin, were selected for the study. The Grassland was dominated by the perennial resprouting grass, *Brachypodium retusum* (Pers.) Beauv. In Mixed Shrub-Grassland the small leguminous shrub, *Genista scorpius* L. in Lam et DC. was scattered over a grassy sward of *B. retusum*. The shrubland was dominated by the evergreen obligate seeder, *Rosmarinus officinalis* L. The study areas had not been subject to fire or grazing by domestic animals for at least 10 years.

Experimental design

Six plots (from 20 to 60 m^2), distributed over different terraces, were selected in each plant community. In October 1996, 12 experimental fires were set in the grassland and mixed-shrub-grassland plots; 21 days later (November) six shrubland plots were burned after cutting all shrubs and letting them dry for a week. Fire intensity was low to medium, with maximum temperatures at ground level being less than 400 °C in grasslands and less than 526 °C in shrublands. Romanyà et al. (2001) described in more detail the soil and fire relationships. Three days after the last fire, a subplot (2×2 m^2) was selected for isotope labelling in a homogeneous burned surface of each plot. The surface of each subplot was labelled with the equivalent of 1 kg N ha^{-1} of $^{15}NH_4Cl$, 99 ^{15}N atom%-excess. To optimise the homogeneity of ^{15}N-enrichment, the surfaces of the subplots were divided into 25×25 cm^2 areas, and each area was sprayed with 18 ml of the labelling solution (1 litter m^{-2}). This application method, as opposed to injection in the root zone, had the advantage of minimal disturbance to the ash layer, while achieving the greatest possible area homogeneity of label. After 24 h, soil sampling recovered 73–81% of the applied $^{15}NH_4^+$ in the top 5 cm of soil. The 80 mm of rainfall in December (measured *in situ* after labelling and prior to plant regrowth) helped to distribute the label in the upper soil volume.

Soil and plant measurements

Plant and soil samples were taken from the 2×2 m^2 labelled burned plots. To avoid boundary effects, we collected legumes and non-legumes (Table 1) from the internal area of each plot (1×1 m^2); the surrounding 25 cm width of each plot was used to take soil samples; the outermost 25 width band was used to estimate plant cover. Soil net N mineralisation was assessed periodically from the autumn burning to early summer using resin-core incubation (DiStefano and Gholz 1986). At each sampling, in each burned plot, we incubated one 5-cm diameter steel cylinder containing the top 5 cm of undisturbed soil and a bag of ion-exchange resin placed at the bottom. Each resin bag contained a mixture of ionic resins (5.8 g of strong cationic macroreticular C-26 Duolite and 5.8 g of strong anionic macroreticular A102D Duolite; BIO-RAD Laboratories, California, USA). An adjacent non-incubated core was also sampled. Daily net N mineralisation rates were calculated as ($N_f + N_r - N_i$)/(incubation days); where N_f is the final post-incubation concentration of mineral N forms ($NH_4{}^+$, $NO_2{}^- + NO_3{}^-$), N_r is the N forms retained by the incubated resins, and N_i is the initial N concentration of the adjacent non-incubated soil samples. Cumulative net N mineralisation over the vegetative period was calculated by summing the net N mineralisation rates from each incubation period.

Resins and soil-exchangeable $NH_4{}^+$ and $NO_3{}^-$ were extracted by shaking with 2 M KCl (1:5 w:v). $NH_4{}^+$ and $NO_2{}^- + NO_3{}^-$ were determined colorimetrically using a Technicon Autoanalyzer (Technicon Instruments Corp. New York, USA). To obtain N per unit area, soil N concentrations were multiplied by the soil mass (fraction < 2 mm) incubated in the first 0–5 cm of the 5-cm

Table 1. Legume and non-legume species growing in burned old-fields used to estimate post-fire N_2-fixation. Species nomenclature and characteristics following Bolòs de and Vigo (1984).

Legume species	Biological types[a]	Habitat[b]	Non-legume species	Biological types[a]	Habitat[b]
Genista scorpius (L.) DC. in Lam. et DC.	NP.	grl & shr	*Sanguisorba minor* Scop.	H.	grl
Ononis pusilla L.	Ch.	grl & shr	*Helianthemum oelandicum* (L.) DC. in Lam. et DC.	Ch.	shr
Ononis natrix L.	Ch.	grl	*Polygala rupestris* Pourr.	Ch.	grl
Ononis spinosa L.	Ch.	grl	*Coris monspeliensis* L.	Ch.	grl & shr
Medicago minima (L.) Bartol	Th.	grl	*Ajuga chamaepitys*(L.) Schreb.	Th.	of
Coronilla scorpioides (L.) Koch	Th.	of	*Teucrium polium* L.	Ch.	shr
Dorycnium pentaphyllum Scop.	Ch.	grl & shr	*Rosmarinus officinalis* L.	NP.	shr
Hippocrepis comosa L.	Ch.	grl & shr	*Salvia verbenaca* L.	H.	grl
Psoralea bituminosa L.	H.	grl	*Lithospermum fruticosum* L.	Ch.	shr
			Plantago sempervirens Crantz	Ch.	grl
			Galium lucidum All.	H.	grl
			Brachypodium retusum (Pers.) Beauv.	Ch.	grl
			Dactylis glomerata L.	H.	grl

[a] Raunkiaer biological type: Th., Therophyta; H., Hemicryptophyta; Ch., Chamaephyta; NP., Nanophanerophyta.

[b] Typical Mediterranean habitat where the species usually grows: grl., grassland; shr., shrubland; of, old-fields.

diameter steel cylinder. In labelled burned subplots, the changes in the isotopic composition of mineral N forms in the top 5 cm of soil were followed for the post-fire 9 months (from autumn to early summer). After addition of MgO to soil extracts and blanks, NH_4^+-N was converted to NH_3 and recovered by diffusion onto acidified filter paper disks sealed between two strips of Teflon [polytetrafluoroethylene (PTFE)] tape (Stark and Hart 1996). Prior to diffusion, soil extract NO_3^- was reduced to NH_4^+ using Devarda's alloy. The NH_3 obtained was analysed by mass spectroscopy (see below) for ^{15}N-enrichment.

In each burned plot, plant cover was monitored on four permanent rectangles (50 × 25 cm) disposed in the outer band 25 cm width of the labelled subplot. On each band we recorded all of the species which touched a 4 mm-diameter pin on a grid of 5-cm intervals. Plant N content and plant ^{15}N-enrichments were calculated by sampling legumes and non-legumes growing in close proximity (< 0.5 m) for later comparison of the ^{15}N-enrichments and N contents; this sampling scheme minimized spatial variability (Shearer and Kohl 1986). Seedlings and resprouts of legumes and non-legumes were collected in each season after fires during the first vegetative period (February, 5; March, 20 and June, 30). Total aboveground biomass was sampled; belowground biomass was not sampled in order to avoid contamination with the ^{15}N-enriched soil-N and, in consequence, later calculations were based on the justifiable assumption that above- and belowground parts had the same isotopic ratio. Soils and plants were ball-milled (MM200 Retsch®) and analysed for %N and isotopic composition at Scottish Crop Research Institute using an ANCA coupled to a model 20-20 Europa isotope ratio mass spectrometer (PDZ–Europa, Crewe, England).

Legume N_2-fixation calculations

The percentage of plant N derived from the atmosphere (%Ndfa) was determined by ^{15}N-enriched isotope dilution (Equation (1); McAuliffe et al. 1958):

$$\%Ndfa = \left[1 - \frac{^{15}N \text{ atom \% excess in the legume}}{^{15}N \text{ atom \% excess in other sources}}\right] * 100 \qquad (1)$$

To estimate the isotope composition of N derived from non-atmosphere sources, we used the ^{15}N-enrichments of two independent references, soil N mean pool abundance (Barraclough 1991) and non-legumes (Shearer and Kohl 1986). The former method estimates the ^{15}N-enrichment of the soil N pool available during the growing period of the plants sampled. The temporal decline of soil available ^{15}N due to the introduction of non-enriched N derived from soil organic matter mineralisation was described by first-order kinetics of the general form (Witty 1983):

$$E_t^* = E_0^* \cdot e^{-Kt} \qquad (2)$$

where E^*_t and E^*_0 are the ^{15}N-enrichments (^{15}N atom % excess) of soil-N at times t and 0, respectively; k is the first-order rate constant (day^{-1}); and t is time (day). Mean pool abundance (E^*) was defined by the integral:

$$\begin{aligned} E^* &= \int_1^2 E_t^* dt/(t_2 - t_1) \\ &= E_0^*(e^{-kt1} - e^{-kt2})/[k(t_2 - t_1)] \end{aligned} \tag{3}$$

where E^* represents the ^{15}N-enrichment of the soil N forms available to plants growing during the time interval from t_1 to t_2. The constants E^*_0 and k, were estimated by fitting for each subplot the temporal decline of ^{15}N-enrichment in the upper 5 cm of soil. Using these parameters with equation 3, we estimated the ^{15}N-enrichment of the mineral N pool (NH_4^+, NO_2^- + NO_3^-) which was potentially available to plants (E^*) for each sampling date (Chalk et al. 1996). Because it was not possible to determine the form of plant available N assimilated by plants, we estimated the ^{15}N-enrichment of the mineral-N pool by a mass-balance equation using the isotopic signature and the amount of N in each extractable mineral form (NH_4^+, NO_2^- + NO_3^-) in each soil sample. For using soil N mean pool abundance, it was assumed that the ratio obtained by integrating the temporal decline of ^{15}N-enrichment in the top 5 cm of soil was similar to the isotopic ratio of the soil N used by legumes and non-legumes. This approach estimated the available ^{15}N-enrichment only in the top 5 cm of soil. The plants would have also accumulated some non−^{15}N-enriched N from soil pools deeper than 5 cm; hence our calculations unavoidably overestimated %Ndfa.

The second approach calculated the %Ndfa of each legume comparing their enrichment (^{15}N atom % excess) with the enrichment of several non-leguminous reference plants (Table 2). Legume seedlings and resprouts were compared against non-legume seedlings and resprouts, respectively. This method assumed similar N exploitation of other sources by legumes and non-legumes. The accuracy of the %Ndfa estimate would be affected to the extent that legumes and non-legumes exploited different soil depths for N. The accuracy would also be affected by the extent to which resprouting legumes used more or less N derived from roots. For this reason, the mean and standard error of %Ndfa was calculated from estimates for individual plants and using in each plot from 1 to 4 reference plants of different species. The reference plants were chiefly non-leguminous forbs and two grasses having different root architectures and, therefore, presumably N uptake strategies. Additionally, as different ^{15}N-natural abundance and higher N content of legume seeds may produce a N-fixation overestimation in early stages of legume seedlings, the %Ndfa estimates were corrected by allowing for the ^{15}N natural abundances and amounts of seed-N in each plant type. We assumed that all seed-N was recovered in the plant and in a ratio proportional to a root-shoot ratio of 0.7. Seed-N, isotopic signature and root:shoot ratio were estimated from samples collected throughout the experimental area (data not shown).

Table 2. Aboveground ^{15}N-enrichment (^{15}N atom% excess) of seedlings and resprouts of legumes and non-legumes collected after fires in each plant community. Mean ± (SE).

	[a]Winter		Early Spring			Summer		
	Grassland	Mixed Shrub-grassland	Grassland	Mixed Shrub-grassland	Shrubland	Grassland	Mixed Shrub-grassland	Shrubland
Legume Seedlings								
Genista scorpius				0.0392 (0.0142)			0.0103 (0.0032)	
Coronilla scorpioides			0.0348 (0.0238)					
Hippocrepis comosa				0.32700 (0.2519)				
Medicago minima				0.3677				
Ononis pusilla				0.6077			0.0417	
Ononis spinosa						0.0077		
Psoralea bituminosa			0.0747	0.0707 (0.0246)	0.6155 (0.1848)	0.0023 (0.0023)	0.0082 (0.0042)	0.0417 (0.0099)
Non-Legume Seedlings								
Ajuga chamaepitys								0.6415
Helianthemum oelandicum					1.2708 (0.1645)			1.1009
Lithospermum fruticosum							0.1090	
Coris monspeliensis				0.5095				
Polygala rupestris								0.5126 (0.0194)
Rosmarinus officinalis					0.6603 (0.1643)			0.3333 (0.0515)
Sanguisorba minor			0.1117 (0.0094)	0.2001 (0.0292)	0.9827 (0.4830)	0.1776 (0.0596)	0.1160 (0.0228)	0.1520 (0.0245)

Legume Resprouts								
Dorycnium penthapyllum								0.0187
Genista scorpius				0.0066 (0.0014)			0.0094 (0.0033)	0.0267
Ononis pusilla			0.0778		0.4168			0.0379 (0.0041)
Ononis natrix						0.0160 (0.0098)		
Psoralea bituminosa	0.0865 (0.0246)	0.3236	0.0320 (0.0107)	0.0861 (0.0365)	0.0567	0.0054 (0.0018)	0.0128 (0.0037)	0.1166 (0.0193)
Non-legume Resprouts								
Brachypodium retusum	0.1603 (0.0154)		0.1389 (0.0188)	0.1935 (0.0502)	1.1033 (0.2559)	0.1389 (0.0178)	0.1190 (0.0081)	0.3976 (0.0680)
Dactylis glomerata	0.5292 (0.0472)	0.5994	0.4504 (0.01034)			0.1949 (0.0178)		
Galium lucidum			0.2404		0.7698 (0.1076)	0.1310 (0.0170)	0.0964 (0.0324)	0.0926
Lithosperumum fruticosum						0.0624	0.0815	
Plantago sempervirens						0.0807		
Salvia verbenaca			0.1543					
Sanguisorba minor	0.3348		0.1859 (0.0928)	0.3246 (0.0947)	0.8866	0.1918 (0.0179)		

[a] In winter, no plants were collected from shrublands.

Statistical analysis

Net N mineralisation and plant cover were distributed normally. The rate of decline of the mineral N pool (*k* parameter) and the intersection (E^*_0 parameter) of the temporal decline of soil available mineral N enrichment model and %Ndfa were log-normal. We used a One-Way analysis of variance (ANOVA) to test differences in soil net N mineralisation between plant communities in each incubation period. The significance of plant cover was tested by a general linear model (GLM) taking the sampling season, plant community and plant regeneration form as fixed factors. After transformation of the data, differences between communities in the *k* and E^*_0 parameters were tested using One-Way ANOVA. When significant, multiple comparisons were done between different levels of each factor using the Least-Significant Difference (LSD) test.

To compare the effects of different estimates of ^{15}N-enrichment derived from non-atmospheric sources (soil mean pool abundance and non-legumes) on %Ndfa, GLM-Repeated Measures was used. After data transformation, non-atmospheric sources estimates were used as within-factor; between factors were sampling season, plant communities and plant regeneration form (seedling or resprout). Because the data failed the sphericity test, the Epsilon corrected averaged *F* of the Greenhouse–Geisser test option was used. All statistical analyses were done using SPSS vs. 9.0.

Results

Soil N availability

During the post-fire 9 months, all communities accumulated about 2.6 ± 0.3 g m^{-2} (mean ± S.E. of 18 plots) net mineralised N. On a smaller time–scale there were differences between communities (Figure 1). After the immediately post-fire burst of net N mineralisation, both grasslands showed net N immobilisation at the end of autumn (November–December), while the shrubland remained in net-N mineralisation. From winter to early summer, net N mineralisation followed similar trends in all burned communities. In early spring, the flux of N oscillated from the net N-immobilisation in March to the highest availability of the studied period in April. In late spring, N availability was lower than winter and similar to that in autumn.

Due to net N mineralisation of unlabelled soil organic matter, soil available $^{15}NH_4^+$-enrichment decreased sharply during the first post-fire month (Figure 2). This dilution of the labelled pool was followed by an increase of $^{15}NO_3^-$-enrichment in both grasslands, but not in the shrubland. During the 9 post-fire months ^{15}N-enrichment of the soil available mineral N pool (NH_4^+ + NO_3^-) decreased exponentially (Figure 2; Table 3). No significant differences were found between communities for the rate of decline of the mineral N pool (*k* parameter) and only slight differences in the intersection

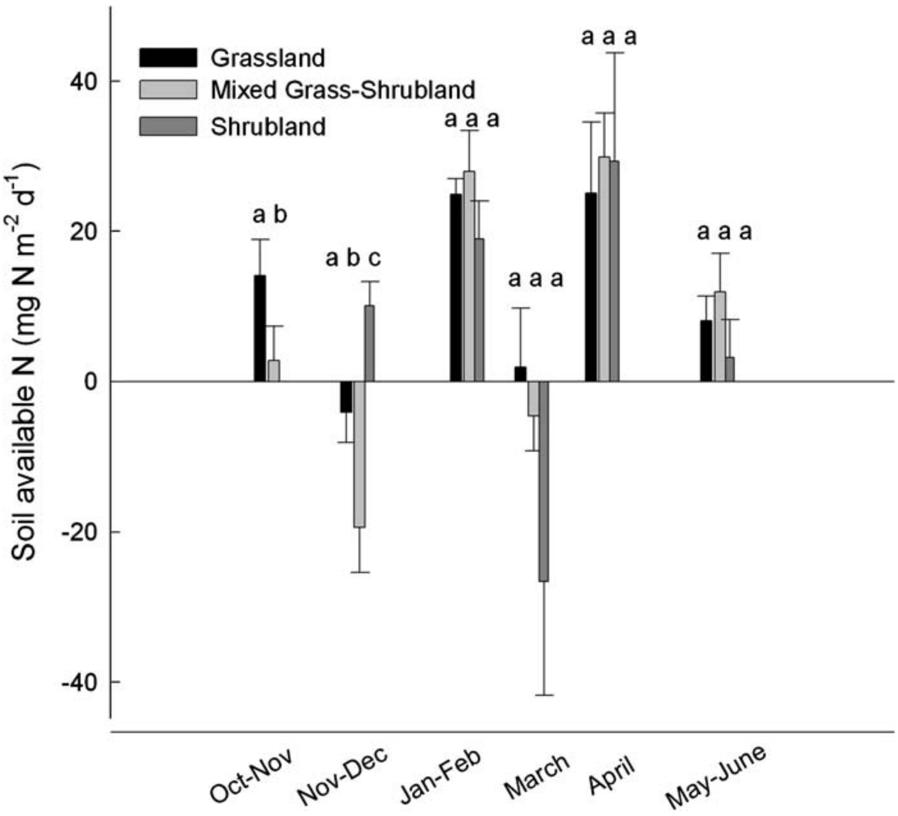

Figure 1. Net N mineralisation and immobilization in the top five cm of soil from each burned plant community studied during the first nine months after fire. Different lower-case letters indicate significant differences among plant communities. Mean ± S.E. ($p < 0.05$; $n = 6$).

parameter (E^{*}_{0}). The integral of these exponential curves (E^{*}) estimated the ^{15}N-enrichment of the soil N mineral forms available to plants growing from fires to each sampling data (winter, spring or summer; Table 3).

Plant cover

In the burned plots, plant regeneration by seedlings and resprouts occurred in the second post–fire month and coincided with the beginning of winter; active growth began towards the end of winter (Figure 3). By early summer there was 63 ± 4% plant cover in *Grassland*, 64 ± 7% in the *Mixed Shrub–Grassland* and 46 ± 5% in *Shrubland*. In both grasslands, plant recovery was mainly due to resprouters (e.g. *B. retusum*); the contribution by both strategies was more equal in shrublands. In this community, resprouter cover was less than 30%. Seedlings were poised to fix N_2 very soon after fire. At 1 to 3 months after fire,

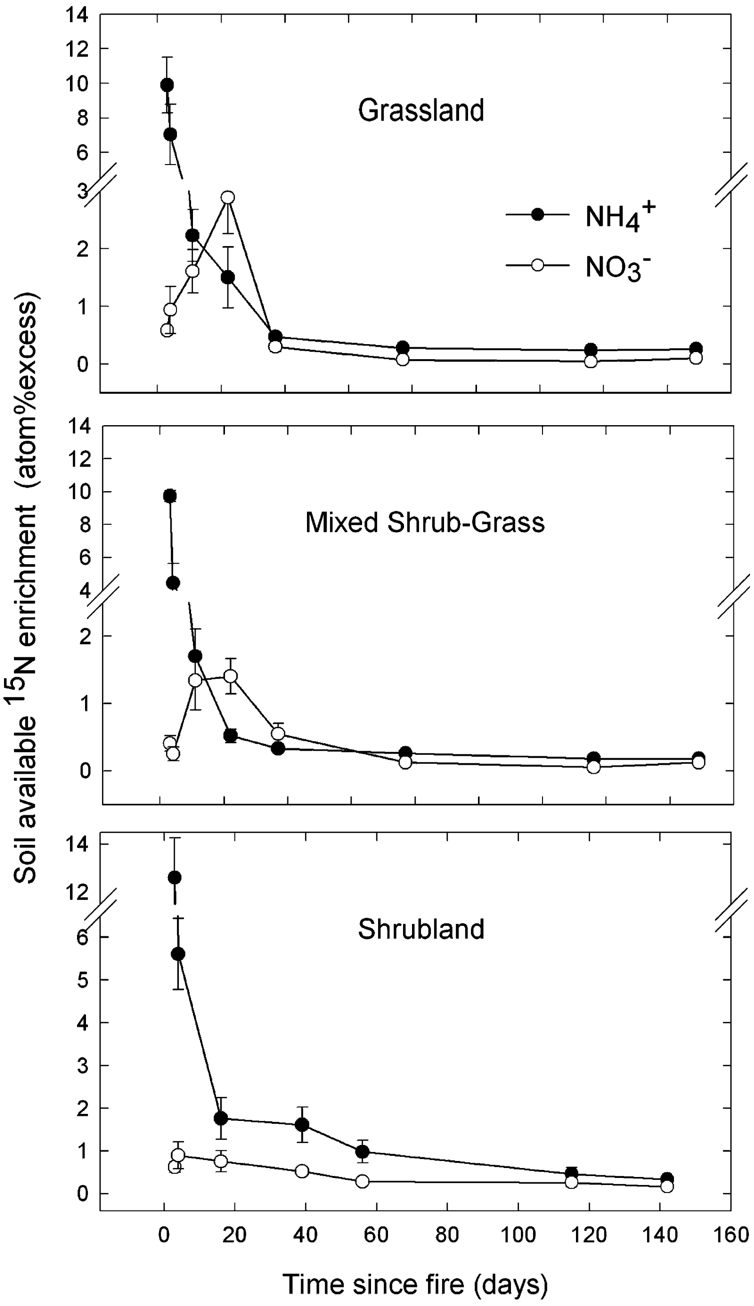

Figure 2. Change in soil $^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$ after fire in the three communities studied. Mean ± S.E. (n = 6).

Table 3. Parameters of temporal ^{15}N enrichment decline equation ($E^*_t = E^*_0 . e^{-kt}$) of soil mineral-N pool in each plant community and 'Mean abundance pool (E^*)' estimated as the integral of each decline equation. Mean ± SE of parameters and significance of equation. Different capital letters indicate differences for intersection parameter (E^*_0, $p < 0.05$); no differences among slope parameter (k) were found ($p = 0.56$).

Plant Community	Parameters of ^{15}N-enrichment decline Equation[a]		Significance			Mean abundance pool (E^*)[b] (^{15}N atom %excess)		
	E^*_0	K	$_{adj}R^2$	*p-value*	n	Winter	Spring	Summer
Grassland	2.22 ± 0.42A	0.0178 ± 0.002A	0.63	*0.001*	42	0.9381	0.7306	0.4588
Mixed Shrub-Grass	1.55 ± 0.36B	0.0169 ± 0.002A	0.68	*0.001*	42	0.6778	0.5317	0.3367
Shrubland	2.92 ± 0.51A	0.0191 ± 0.002A	0.70	*0.001*	36	1.3751	1.0167	0.6084

[a] $E^*_t = E^*_0 . e^{-kt}$ where E^*_t and E^*_0 refer to the mineral N pool ^{15}N enrichment (atom % excess) of the upper 5 cm of soil at day t and day 0 after fire, respectively; and k, the declining constant.

[b] ^{15}N-enrichment of available soil mineral N forms from fires to each sampling date estimated as $E^* = \int_1^2 E^*_t dt/(t_2 - t_1) = E^*_0(e^{-kt1} - e^{-kt2})/[k(t_2 - t_1)]$.

legume seedlings in all plant communities had cotyledons, 2 to 5 leaves and 1 to 5 root nodules containing red pigment.

N_2-fixation using non-legumes and mean soil abundance pool as references

The percentage of plant N derived from the atmosphere (%Ndfa) was obtained by isotope dilution in legume seedlings (Appendix 1) or resprouts (Appendix 2) using either several seedlings or resprouts of non-legumes and the soil mean abundance pool as non-fixing comparators with which to calculate the ^{15}N-enrichment derived from non-atmospheric sources. ^{15}N-enrichment (^{15}N atom % excess) clearly differentiated legumes and non-legumes in spring and summer (Table 2). Non-legumes' ^{15}N-enrichment was close to the ^{15}N-enrichment of the mean abundance pool of available N in the first 5 cm of soil (Tables 2 and 3), and legume ^{15}N-enrichment was close to the atmospheric value (atmosphere enrichment = 0 ^{15}N-atom % excess).

The %Ndfa using both types of estimations was highly correlated ($_{adj}r^2 = 0.91$, $p < 0.001$, $n = 81$), but the calculations using the soil mean abundance were 8% higher than those using non-legumes (t-paired test, $p < 0.001$, $n = 81$). Plant uptake from the less ^{15}N-enriched soil underneath the 0–5 cm layer could have overestimated reliance on atmospheric N_2 when the soil mean abundance pool method was used. Using both methods, two meaningless results, with values lower than 0, were obtained for legume-seedlings (Appendix 1), probably because of legume contamination with ^{15}N-enriched soil. In each legume individual, the %Ndfa estimated by different non-legume reference species was of similar magnitude: the coefficient of variation (%) for the %Ndfa of 33 estimates ranged from less than 1–51% with a

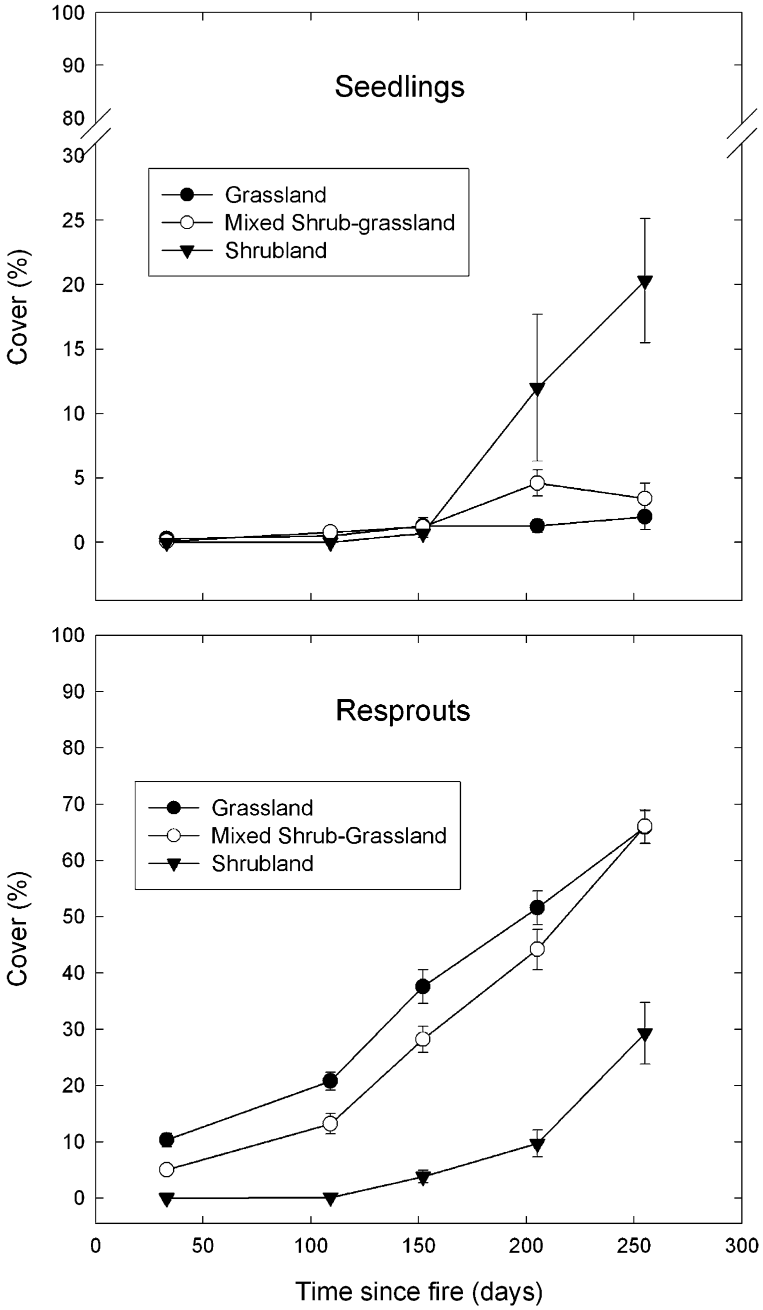

Figure 3. Seedling and resprout plant cover in the three communities studied during the first nine months after fire. Mean ± S.E. (n = 6).

Table 4. Proportion of legume-N derived from atmosphere (%Ndfa ± SE) measured by isotopic dilution, using non-legume reference plants or mineral-N mean abundance pool as independent estimates of the ^{15}N-enrichment derived from soil. Significant differences of the repeated measurements test of within and between subjects are indicated.

Sampling Season	Plant regen. forms	Non-Legumes			Mineral-N Mean Abundance Pool		
		Grassland	Mixed Shrub-Grass	Shrubland	Grassland	Mixed Shrub-Grass	Shrubland
Winter							
	Resprouts	76 ± 5	48		91 ± 3	52	
Spring							
	Seedlings	84 ± 6	68 ± 10	63 ± 8	93 ± 3	82 ± 5	58 ± 9
	Resprouts	78 ± 7	81 ± 9	73 ± 19	95 ± 2	91 ± 4	77 ± 18
Summer							
	Seedlings	97 ± 0	90 ± 3	83 ± 3	99 ± 0	97 ± 1	93 ± 1
	Resprouts	92 ± 4	89 ± 4	90 ± 2	98 ± 1	97 ± 1	91 ± 3
	Within Subjects			*p-value*			
	soil enrichment estimates			0.001			
	soil enr. estimates × Plant Community			0.03			
	Between Subjects						
	plant community			0.002	Grassland, mixed > shrubland		
	sampling season			0.001	winter, spring < summer		
	plant regeneration form			0.250	Seedlings, resprouts		

median of 3.8%. Some reference species, mainly *B. retusum* sprouts, gave lower values than the average of estimates using the rest of non-legumes: the average of %Ndfa estimates using *B. retusum* was 3.7% ± 1.3% lower ($p = 0.01$, $n = 24$, t-paired test) than the estimates obtained without this reference plant. *B. retusum* is a perennial grass with extensive branched rhizomes mostly located between 2–10 cm soil depth and which resprouts quickly after fire.

The %Ndfa of different legume species ranged from 52 to 99%, depending on the sampling season and plant community (Table 4). In each collection season, using both reference methods, there was slightly lower %Ndfa for shrubland legumes than for Grassland or Mixed Grassland (plant community $p = 0.002$). %Ndfa increased from spring to summer. In spring, Ndfa was about 75% in both grasslands and 68% in shrublands, and increased in early summer to 90% in grasslands and 87% in shrublands (Table 4). There was no interaction between season and plant community. There were no significant differences between legume species or between seedlings and resprouts for any set of species (Table 4) or for any single species (Table 5). Thus, comparing seedlings and resprouts of the two most abundant legume species in the mixed shrub-grass community, the small shrub *Genista scorpius* and the herb *Psoralea bituminosa*, no differences existed between species or plant regeneration forms (Table 5).

Discussion

N_2 -*fixation and soil N availability*

The N derived from fixation (%Ndfa) for different legume seedlings and resprouts in the 9 months post-fire was 52–99%. The %Nfda's obtained in this study were comparable with those of *Ceonothus* and *Purshia* shrubs growing in a wide range of overstory stand conditions in central Oregon pine forests

Table 5. Proportion of legume-N derived from atmospheric-N (%Ndfa ± SE; and number of cases, (n) in the legume shrub, *Genista scorpius*, and in the herbaceous legume, *Psoralea bituminosa*, growing in the mixed shrub-grass community measured by the isotopic dilution method using non-legume reference plants. The significance of tested factors (*p-value*; GLM test) is indicated; non significant interactions, *p-value* > 0.1, are not indicated.

Sampling Season	*Genista scorpius*		*Psoralea bituminosa*	
	Seedlings	Resprouts	Seedlings	Resprouts
Spring	68 ± 12 n = 8	79 ± 12 n = 4	52 ± 17 n = 4	64 ± 12 n = 6
Summer	91 ± 4 n = 3	87 ± 6 n = 11	90 ± 5 n = 5	84 ± 6 n = 3
	factors	*p-value*		
	legume species	0.34		
	plant regeneration form	0.76		
	sampling season	0.01		

(Busse 2000). Using acetylene reduction and nodule biomass, Hendricks and Boring (1999) also reported high N_2-fixation in herbaceous legumes after fire in Southeastern United States. In the present study, two independent estimates for N derived from non-atmospheric sources gave similar %Ndfa estimates. In addition, the narrow range of %Ndfa's which were calculated independently, using different non-legumes, corroborate the robustness of the isotope dilution method to reference plant selection in non-uniformly labelled soil when N_2-fixation is strong (Danso et al. 1993; Busse 2000).

The cumulative net N mineralisation during the post-fire nine months (2.6 g Nm^{-2} in the top 5 cm of soil) was comparable with amounts reported for tallgrass prairies after fire (Ojima et al. 1994; Blair 1997). We measured net N mineralisation only in the top 5 cm; in the entire rooting volume plant available N would have been greater than our measured values. In spite of abundant soil N, %Ndfa accounted for more than 50% of post-fire aboveground legume N contents. Depending on the abundance of legume regeneration after fire, these high fixation rates may contribute to replace N losses produced by fires.

An open canopy combined with a low soil N availability favours N_2-fixation over soil N use because optimal growth is achieved by allocating higher proportion of photosynthates to N_2-fixation rather than allocating toward fine roots to acquire soil N (Rastetter et al. 2001; Vitousek et al. 2002). In the present study regenerating vegetation appeared to rely heavily on fixed N_2 for the first six months after fires. Cover estimates (lower than 40–50%) verified that the canopy was open during this period, and light competition was, therefore, minimal in this high-light environment. From early spring to summer, %Ndfa continued to increase, and conditions more nearly matched those commonly associated with large reliance on N_2-fixation, i.e. low soil N availability (net N mineralisation declined), increased competition for soil N (as suggested by increasing cover) and high light penetration in this Mediterranean region. In addition, slightly lower %Ndfa of legumes growing in shrublands might be interpreted as a lower competition for soil N related to lower root density in shrublands in comparison with grasslands where well-established grass resprouters, mainly *B. retusum*, dominated after fires. Then, despite similar soil N mineralisation in all studied plant communities, higher competition for soil resources between legumes and non-legumes may have increased the ratio of N_2-fixation as observed in grasslands.

N_2 -fixation by legume seedlings and resprouts

In the plant communities studied, legume seedlings and resprouters equally relied on large amount of fixed N_2 during the first vegetative post-fire period. This result do not support the hypothesis, based on the Multiple Element Limitation model (Rastetter et al. 2001; Vitousek et al. 2002), that states that a well-developed root system of legume resprouts in comparison with seedlings has a great effect on N_2-fixation rates. There was no difference in %Ndfa between legume seedlings or resprouts, even when comparing both regenerating forms of a single species. The %Ndfa for seedling legumes was higher than that found by Hansen et al. (1987), using the acetylene reduction, in S.W. Australia, where legume seedlings growing in burned *Eucalyptus marginata* forests fixed 13–61% of plant N in the first post-fire year.

In the communities which we studied, legume seedlings with only cotyledons and 2–5 leaves were nodulated and had, after seed-N correction, large %Ndfa. Large amounts of net N mineralisation in the top 5 cm of soil, where these legume seedlings had most of their root systems, did not inhibit N_2-fixation. Seedling survival in Mediterranean communities were partially explained by the ability to allocate resources to belowground vs. aboveground organs and this pattern was apparently associated with the ability to take up, in addition to water, soil nutrients (Lloret et al. 1999). Our results suggest that both legume regeneration forms, seedlings and resprouts, allocated more resources to N_2-fixation than to N uptake short-term after fires.

In conclusion, according to Vitousek and Field (1999) simulations, the conditions during the first revegetative period after low or medium intensity fires, high light availability apparently favoured N_2-fixation despite the high soil N mineralisation. High fine root densities in grasslands dominated by *B. retusum* grass might explain slightly higher N_2-fixation rates of legumes growing in both grasslands than in shrublands. Opposite to our hypothesis, both legume seedlings and resprouts had high fixation rates during this period. Different root density and architecture between these plant regeneration forms do not produce significant differences on N_2-fixation rates. In agreement with their 'high N-rich lifestyle' (Mckey 1994), legumes that regenerated short term after fire showed a high N_2-fixation rates and may play an important role in replacing N lost by fires. More research is necessary to evaluate N_2-fixation for longer periods of time in order to assess post-fire recovery of ecosystem productivity.

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Appendix 1. Proportion of legume seedling N derived from atmosphere (%Ndfa) as estimated by isotope dilution and using non-legume seedlings[e] or soil mean abundance pool (soil E) as estimators of ^{15}N derived from non-atmosphere sources.

Site[a]	Legume Species Characteristics					Ndfa estimates									
						Using non-legumes[e]								Soil estimates	
	Species	Phe[b]	Pl.W.[c] mg	N %	^{15}N[d] at%exc	Sm %	Ho %	Ro %	Lf %	Ac %	Pr %	Cm %	Ndfa Mean ± SE	Soil E at%exc	Ndfa %
Early Spring															
B1	*Coronilla scorpioides*	c5L	46	2.60	0.0657	79							79	0.731	91
B2	*Coronilla scorpioides*	c5L	39	2.76	0.0127	96							96	0.731	98
B3	*Psoralea bituminosa*	c4L	17	2.40	0.0747	76							76	0.731	90
G1	*Genista scorpius*	c3L	15	1.76	0.2067	34							34	0.532	61
G1	*Psoralea bituminosa*	c3L	17	2.40	0.1657	27							27	0.532	69
G3	*Genista scorpius*	c4L	15	2.57	0.0997	68							68	0.532	81
G4	*Genista scorpius*	c5L	20	2.00	0.0397	85							85	0.532	93
G4	*Psoralea bituminosa*	c6L	20	2.42	0.0717	73							73	0.532	86
G6	*Genista scorpius*	c4L	9	2.25	0.0787							85	85	0.532	85
G6	*Genista scorpius*	c5L	9	3.70	0.0117							98	98	0.532	98
R1	*Psoralea bituminosa*	c4L	30	2.79	0.2717	71							71	1.017	73
R2	*Psoralea bituminosa*	c5L	20	3.46	1.2747	− 37							−37	1.017	−25
R2	*Hippocrepis multisiliq.*	c5L	39	4.97	0.6077	35							35	1.017	40
R2	*Medicago minima*	c6L	19	4.22	0.3677	61							61	1.017	64
R3	*Hippocrepis multisiliq.*	c5L	14	4.46	0.0877	89	92	93					91	1.017	91
R4	*Psoralea bituminosa*	c3L	13	3.17	1.2427			− 119					−76	1.017	−22
R5	*Ononis pusilla*	c5L	15	3.23	0.6077		59						59	1.017	40
R5	*Psoralea bituminosa*	c5L	18	2.89	0.5997		60						60 ± 1	1.017	41
Summer															
B2	*Psoralea bituminosa*	V	160	2.06	0.0077	97							97	0.459	98
B2	*Ononis spinosa*	V	768	1.01	0.0077	97							97	0.459	98
B4	*Psoralea bituminosa*	VF	1446	1.79	−0.0043	103							103	0.459	101

Appendix 1. Continued.

Site[a]	Legume Species Characteristics					Ndfa estimates									
						Using non-legumes[e]								Soil estimates	
	Species	Phe[b]	Pl.W.[c] mg	N %	^{15}N[d] at%exc	Sm %	Ho %	Ro %	Lf %	Ac %	Pr %	Cm %	Ndfa Mean ± SE	Soil E at%exc	Ndfa %
B4	*Psoralea bituminosa*	V	209	2.38	0.0037	97							97	0.459	99
B6	*Psoralea bituminosa*	V	879	2.27	0.0027	99							99	0.459	99
G1	*Psoralea bituminosa*	V	278	2.14	0.0037	97							97	0.337	99
G1	*Genista scorpius*	V	295	3.03	0.0097	92							92	0.337	97
G1	*Psoralea bituminosa*	V	148	1.53	0.0107	90							90	0.337	97
G3	*Psoralea bituminosa*	V	204	2.08	0.0287	72							72	0.337	92
G3	*Genista scorpius*	V	317	0.88	0.0177	83							83	0.337	95
G4	*Genista scorpius*	V	578	2.11	0.0047	98							98	0.337	98
G6	*Psoralea bituminosa*	c4L	107	2.38	0.0097				91				91	0.337	97
R1	*Psoralea bituminosa*	V	177	2.60	0.0487	66					90		78 ± 8	0.608	92
R2	*Psoralea bituminosa*	V	199	2.63	0.0777			82			85		84 ± 1	0.608	87
R3	*Psoralea bituminosa*	V	245	2.57	0.0147	93		97		98	97		96 ± 1	0.608	98
R4	*Psoralea bituminosa*	V	886	2.70	0.0387	72		87					80 ± 5	0.608	94
R4	*Ononis pusilla*	VF	528	1.65	0.0417	70		86					78 ± 6	0.608	93
R4	*Psoralea bituminosa*	VF	930	1.66	0.0337	76		89					82 ± 5	0.608	94

[a] Site Reference: *B. Brachypodium retusum* Grassland; *G.* Mixed *Genista scorpius* Shrub-Grassland; *R. Rosmarinus officinalis* Shrubland. Numbers refer to plots.

[b] Phe = Plant phenology: *C.* cotyledons and number of leaves; *V.* Vegetative , *VF* Flower + Fruit.

[c] Pl W.: total plant weight adding belowground weight estimated as 0,4 of aboveground weight.

[d] ^{15}N atom % excess corrected by legume seed weight.

[e] %Ndfa estimates using individuals of non-legumes growing near each legume plant: Sm. *Sanguisorba minor*; Ho. *Helianthemum oelandicum*; Ro. *Rosmarinus officinalis*; Lf. *Lithospermum fruticosum*; Ac. *Ajuga chamaepytis*; Pr. *Polygala rupestris*; Cm. *Coris monspeliensis*.

Appendix 2. Proportion of legume resprout N derived from atmosphere (%Ndfa) as estimated by isotope dilution and using non-legume resprouts[c] or soil mean abundance pool (soil E) as estimators of the ^{15}N derived from non-atmosphere sources.

Site[a]	Legume Species Characteristics				Ndfa estimates										
					Using non-legumes[c]									Soil estimates	
	Species	Phe[b]	N %	^{15}N at%exc	Br %	Sm %	Dg %	Gl %	Ho %	Lf %	Ps %	Sv %	Ndfa Mean ± SE	Soil E at%exc	Ndfa %
Winter															
B5	*Psoralea bituminosa*	V	3.43	0.0677	45		88						66 ± 15	0.938	93
B6	*Psoralea bituminosa*	V	3.38	0.0567	69	82	88						80 ± 5	0.938	94
G6	*Psoralea bituminosa*	V	2.82	0.1357			82						82	0.938	86
B6	*Psoralea bituminosa*	V	3.82	0.3237			46	50					48 ± 1	0.678	52
Early Spring															
B2	*Psoralea bituminosa*	V	2.81	0.0527	57		78					66	67 ± 5	0.731	93
B3	*Ononis natrix*	V	3.79	0.0777	36		68		50			50	51 ± 8	0.731	89
B5	*Psoralea bituminosa*	V	3.12	0.0547		60	83	77					73 ± 6	0.731	93
B5	*Psoralea bituminosa*	V	3.86	0.0003	100	100							1100 ± 0	0.731	100
B6	*Psoralea bituminosa*	V	3.95	0.0147	85	95							90 ± 3	0.731	98
G1	*Genista scorpius*	V	3.80	0.0387	73		94						84 ± 8	0.731	95
G1	*Psoralea bituminosa*	V	4.08	0.0067	95								95	0.532	99
G2	*Genista scorpius*	V	4.47	0.0147	90								90	0.532	97
G3	*Psoralea bituminosa*	V	4.68	0.0047		98							98	0.532	99
G4	*Genista scorpius*	V	3.73	0.1627	16	50							33 ± 12	0.532	69
G4	*Psoralea bituminosa*	V	4.39	0.0107	96	97							97 ± 1	0.532	98
G6	*Psoralea bituminosa*	V	5.12	0.0337	86	92							89 ± 2	0.532	94
G6	*Psoralea bituminosa*	V	3.44	0.1337	31	59							45 ± 10	0.532	75
R1	*Genista scorpius*	V	4.18	0.0037	98	99							98 ± 0	0.532	99

Appendix 2. Continued.

Site[a]	Legume Species Characteristics				Ndfa estimates										
					Using non-legumes[c]									Soil estimates	
	Species	Phe[b]	N %	^{15}N at%exc	Br %	Sm %	Dg %	Gl %	Ho %	Lf %	Ps %	Sv %	Ndfa Mean ± SE	Soil E at%exc	Ndfa %
R1	*Psoralea bituminosa*	V	4.01	0.567	93			91					92 ± 1	1.017	94
R5	*Ononis pusilla*	V	3.91	0.4167	62	53		46					54 ± 1	1.017	59
Summer															
B2	*Ononis natrix*	V	1.70	0.0257	76		80						78 ± 2	0.459	94
B3	*Psoralea bituminosa*	V	1.91	0.0077	91	96	97	94					95 ± 1	0.459	98
B3	*Ononis natrix*	FL	1.66	0.0067	93	97	98	95					96 ± 1	0.459	99
B5	*Psoralea bituminosa*	FL	1.42	0.0017	99	99	99	99		97	98		98 ± 0	0.459	100
B6	*Psoralea bituminosa*	FL	2.08	0.0067	97					90			93 ± 3	0.459	99
G1	*Genista scorpius*	FL	1.27	0.0197	86								86	0.337	94
G1	*Genista scorpius*	V	1.47	0.0177	87								87	0.337	95
G2	*Genista scorpius*	FL	1.33	0.0027	98			98					98 ± 0	0.337	99
G2	*Genista scorpius*	V	0.88	0.0037	97			97					97 ± 0	0.337	99
G3	*Genista scorpius*	FL	1.84	0.0237				63					63	0.337	93
G3	*Psoralea bituminosa*	V	2.17	0.0167				74					74	0.337	95
G4	*Genista scorpius*	V	1.15	0.0037	97								97	0.337	99
G5	*Psoralea bituminosa*	FL	2.11	0.0057	96								96	0.337	98
G5	*Genista scorpius*	V	1.13	0.0037	97								97	0.337	99
G6	*Psoralea bituminosa*	FL	1.99	0.0167	82					80			81 ± 1	0.337	95
G6	*Genista scorpius*	FL	1.73	0.0070	99							93	96 ± 2	0.337	100
R3	*Ononis pusilla*	FR	1.76	0.0387	85								85	0.337	94
R3	*Ononis pusilla*	FR	2.51	0.0447	89			96					92 ± 3	0.608	93

R5	*Psoralea bituminosa*	V	2.92	0.1357		88	88	0.608	78
R6	*Ononis pusilla*	V	1.95	0.0307		97	97	0.608	95
R6	*Genista scorpius*	FL	1.23	0.0267	94		94	0.608	96
R6	*Dorycnium penth*	V	1.86	0.0187	96		96	0.608	97
R6	*Psoralea bituminosa*	FL	2.16	0.0977	80		80	0.608	84

[a] Site Reference: *B. Brachypodium retusum* Grassland; *G.* Mixed *Genista scorpius* Shrub-Grassland; *R. Rosmarinus officinalis* Shrubland. Numbers refer to plots.

[b] Plant phenology: *V.* Vegetative; *FL.* Flower; *FR* Flower + Fruit.

[c] %Ndfa estimates using individuals of non-legume species growing near each legume plant*: Br. Brachypodium retusum; Sm. Sanguisorba minor; Dg. Dactylis glomerata; Gl. Galium lucidum; Ho. Helianthemum oelandicum; Lf. Lithospermum fruticosum; Ps. Plantago sempervirens; Sv. Salvia verbenaca.*

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