Factors affecting the establishment and growth of annual legumes in semi-arid mediterranean grasslands

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Abstract Legumes are an important component of mediterranean grasslands with a significant ecological and economic role. The aim of this study was to investigate the factors that affect their establishment and growth and how they survive in a highly variable and unpredictable environment. The research was carried out in a grassland characterised by a semi-arid mediterranean climate and located on a calcareous substrate at about 150 m a.s.l., in Macedonia, northern Greece. It was dominated by annual legumes such as *Hippocrepis multisiliquosa*, *Medicago disciformis*, *Medicago minima*, *Onobrychis aequindentata*, *Trifolium angustifolium*, *Trifolium campestre* and *Trifolium scabrum*. It was subjected to the following treatments for four consecutive years: prescribed burning, irrigation, digging, cutting, P fertilization and control. Total legume density was measured in late autumn and in the following spring each year, while total legume biomass was measured only in spring. Dominant legume species densities and biomasses were measured only in spring in the last 3 years. Also, monthly precipitation and air temperature were recorded in a nearby weather station. A great reduction of both legume density and biomass occurred at the third growing season due to adverse weather conditions. Among treatments, P fertilization affected the positively annual legume density and biomass. The other treatments such as burning, irrigation, digging and cutting influenced positively or negatively annual legume density and biomass depending on the climatic characteristics of the particular growing season involved. It is concluded that in semi-arid mediterranean grasslands with cold winters, weather conditions strongly interact with human disturbances in affecting establishment and growth of annual legumes.

Keywords Biomass · Burning · Cutting · Density · Digging · Legumes · P fertilization · Weather changes

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Introduction

Grasslands of the mediterranean region cover a relative limited area but constitute an important ecological and economic resource (Papanastasis and Mansat 1996). Their vegetation is dominated by annual species due to the mediterranean climate that is characterised by a wet and cool winter period followed by a hot and dry summer. As a matter of fact, the longer and drier the summer period, the stronger is the seasonability resulting in greater predominance of the annual species (Seligman

1996). These species are well adapted to the highly variable mediterranean climate because they produce high amount of small seeds and have a long-lived seed bank (Teketay 1996; Pagnotta et al. 1997).

Extreme climatic variables (e.g. drought) may restrict the number of grassland species (Tilman and El Haddi 1992). On the other hand, Koukoura and Papanastasis (1997) and Koukoura et al. (1998) have found that legume abundance is correlated with air temperature and precipitation. Similar results are also reported by Fernàndez Alès et al. (1993) in Spain, who found that legume abundance is particularly influenced by spring precipitation.

Besides climate, several other factors are affecting mediterranean grasslands but their impact on species structure is conflicting. Individual grassland species have evolved specific adaptations to burning that allow them to survive periodic fires (Pausas 1999; Pignatti et al. 2002). Le Houerou (1981) concluded that the structure of mediterranean grasslands after fire is determined by the reaction of every single species as fire not only removes the vegetation but also has a direct effect on germination and seedling survival (Hanley and Fenner 1998). Menke (1989) found low density of grasses after fire while Papavasiliou and Arianoutsou (1993) reported a dominance of leguminous species in recently burnt forest communities.

Grazing in mediterranean grasslands reduces production and affects species composition (Noy-Meir 1998). By increasing grazing intensity, there is a subsequent decrease of grasses and an increase of legume abundance (Naveh and Whittaker 1979). Drought and grazing may interact as defoliation produced by grazing can reduce the plant's ability to sustain drought and vice versa, so drought and grazing are seen as complementary forces (Vesk and Westoby 2001). Grazing also interacts with fire in affecting species composition in mediterranean grasslands (Noy-Meir 1998).

Ploughing is a more serious disturbance than grazing as it totally destroys biomass. Doerr et al. (1984) report that the lighter the soil disturbance, the sooner the grassland species recover. On the other hand, annual legumes are among the first species to colonise abandoned arable fields (Noitsakis et al. 1992).

Fertilization is an effective management practice aiming at quantitative and qualitative improvement of herbage (Menke 1989; Osman et al. 1999).

Phosphorous (P) especially is essential for the legume establishment though it is known that plant response to P fertilization is expected only in wet years (Henkin and Seligman 2000). Generally, P fertilization alters species composition in grasslands by favouring legumes at the expense of grasses (Papanastasis and Koukoulakis 1988; Osman et al. 1999; Henkin et al. 2006).

In arid and semi-arid mediterranean grasslands, species composition is affected more by short-term weather changes than grazing intensities (Tsiouvaras et al. 1998). According to Jackson and Bartolome (2002), variations of species composition and biomass in the annual grasslands of California are associated with weather patterns. In the mediterranean basin, in particular, moisture is the limiting factor affecting primary productivity that fluctuates both within and between years (Le Houérou and Hoste 1977; Naveh 1982; Papanastasis 1982). Irrigation favours annual species but for a very short time, as perennials eventually dominate (Powell et al. 1990).

Annual legumes are an important component of mediterranean grasslands. In semi-arid environments, they may make more than 20% of the total number of species present (Papanastasis 1981). Their importance mainly lies on their ability to fix atmospheric nitrogen, which is the primary functional trait that influences nitrogen accumulation and biomass production (Spehn et al. 2002). In addition, they are superior to grasses in feeding value for animals because they have higher content of protein and minerals resulting in higher voluntary intake and a faster rate of digestion (Van Soest 1994). In this paper, we investigated how fire, soil digging and cutting as well as P fertilization and irrigation affect the establishment and growth of annual legumes in mediterranean grasslands with a semi-arid climate so that their function is better understood and management is properly implemented.

Materials and methods

The research was conducted in a mediterranean grassland of Macedonia, northern Greece (E 23° 58′ 49″, N 41° 11′ 30″) located on a calcareous substrate at about 150 m a.s.l. Long term monthly temperature ranges from 3°C in January to a high of 25°C in July.

Mean annual precipitation is 589.4 mm, and the mean minimum temperature of the coldest month (January) is 0.1°C indicating a semi-arid mediterranean climate with cold winters (Le Houerou 1981). The dry period is from July to September, whereas the driest month is September.

The soil of the area is of medium depth, sandy clay with neutral reaction. It is rich in humus but low in concentration of phosphorous while it is adequately rich in cations.

The grassland was dominated by annuals such as the grasses *Avena barbata* Pott ex Link, *A. sterilis* L. and the legumes *Onobrychis aequindentata* (Sibth. and Sm.) D' Urr., *Trifolium angustifolium* L., *Trifolium campestre* Schreber and *T. scabrum* L. *Hippocrepis multisiliquosa* L., *Medicago disciformis* DC., *Medicago minima* (L.) Bartal., but perennial species were also present with most important being the grass *Dichanthium ischaemum* (L.) Roberty.

In late summer of 1993, a homogenous in species composition area of 30 × 30 m was selected and fenced out in order to be protected from sheep and goats, which were grazing in the whole grassland in a communal way. In this fenced area, six treatments were applied in a completely randomized block design with four blocks. Treatments included prescribed burning, irrigation, digging, cutting, P fertilization and control. They were applied in plots 3 × 3 m in size and they were arranged randomly within each block.

Prescribed burning, P fertilization and digging were applied in early autumn of 1993 and repeated in 1994, 1995 and 1996. The first treatment involved burning of aboveground biomass as an imitation of grassland wildfires often set by shepherds at the end of the summer in order to remove the old growth and encourage the establishment of palatable vegetation after the first autumn rainfall (Papanastasis 1981); the second treatment involved the application of 100 P_2O_5 kg/ha, recommended for favouring legumes in grasslands (Papanastasis and Koukoulakis 1988); and the third treatment involved digging of the soil down to 30 cm with a chopping hoe as an imitation of ploughing for sowing winter cereals in grasslands. The cutting treatment was applied in the middle spring of 1994, during the full bloom period of legumes, by removing the aboveground vegetation as an imitation of grazing and repeated in 1995, 1996 and 1997. Irrigation, finally, was applied both in autumn (November) and spring (March) of all the growing seasons by spreading 30 and 40 mm of faucet water, respectively.

In each treatment, legumes were sampled with quadrats of 0.5 × 0.5 m in size. Within each quadrat, total legume density was measured in late autumn (beginning of December) and the following spring (middle of April) in each of the four growing seasons, by counting the number of individual plants of all legumes present. In spring, the seven dominant species were counted separately. Also, total legume biomass was measured at the end of the growing season (middle of May), by cutting above-ground vegetation within each quadrat and hand separating the legumes from the collected material; the seven dominant species were hand separated only in the last 3 years. In both the measurements five quadrats per plot were taken. The quadrats were taken in different places within each plot every growing season in order to avoid any sampling effect from the previous ones. The non-leguminous species collected in each quadrat are not reported in this study.

In addition, meteorological data were collected from the weather station of the city of Drama, located 10 km away and in the same plain with the study area. They included monthly air temperatures and precipitation.

Density and biomass data were subjected to the analysis of variance both between treatments in a year and between years in a treatment using the software package SPSS. If significant, means were further assessed with the Tukey's test at the 0.05 level of significance (Sokal and Rolf 1981).

Results

Weather conditions

Weather parameters changed widely during the four growing seasons of the experiment. In general, the first two seasons (1993–1094 and 1994–1995) were warmer but drier than the other two (1995–1996 and 1996–1997). More specifically, mean air temperature for the seven months (October–April) of the four growing seasons were 10.2, 12.0, 8.4 and 9.8°C compared with the 20-year value of 9.5°C (1978–1998). For the minimum air temperature, the mean values for the seven months were 5.6, 6.8, 4.1 and 4.9°C, respectively, for the four seasons compared

with the 20-year value of 4.0°C. For the precipitation, finally, the 7-month totals were 345, 277, 466 and 427 mm compared to the 20-year value of 375 mm. These data show that the third growing season (1995–1996) was the coldest but also the wettest. Nevertheless, relatively little rainfall was recorded in the autumn months October (2.6 mm) and November (44 mm) in the third compared with the other three (22 and 65 mm on the average, respectively). At the same two months, mean air temperature was 9.2 and 3.1°C, respectively, compared with the other three (11.7 and 5.9°C on the average, respectively).

Density

Total legume density varied widely among the four growing seasons, between autumn and spring of the same growing season and among the six treatments. More specifically, autumn density was significantly increased from the first to the second growing season in all treatments, but it sharply decreased in the third, to increase again but not significantly in the fourth growing season. This increase, however, did not help reach the density levels of the first two growing seasons. An exception was observed in the P fertilization treatment where legume density was significantly reduced only in the third growing season but remained the same in the other three (Table 1). On the other hand, total legume density was increased from autumn to spring in all four growing seasons, especially in the third (1995–1996). However, spring density was sharply decreased in the third growing season, but it did not recover to the levels of the first two growing seasons except in the P fertilization treatment, as in the autumn season (Table 2).

Table 1 Means of total legume density (plants/m^2) in the autumn in the various treatments for four years

Treatments	1993	1994	1995	1996
Burning	66.5aB	160.0abC	4.2cA	33.2aA
Irrigation	165.3cB	304.0dC	1.0abA	41.2abA
Digging	90.5abB	200.0bcC	1.4abA	32.8aA
Cutting	132.0bcB	242.4cdC	0.2aA	38.4abA
P fertilization	142.5bcB	120.8aB	1.0abA	130.0cB
Control	150.8bcB	289.6dC	2.4bcA	50.0bA

Means of each treatment within the same year followed by the same small letter and between years followed by the same capitals are not significantly different at $p \leq 0.05$

Table 2 Means of total legume density (plants/m^2) in the spring in the various treatments for four years

Treatments	1994	1995	1996	1997
Burning	233.6aB	263.6abcB	26.6bA	55.2aA
Irrigation	238.4aB	270.3bcB	3.4aA	47.2aA
Digging	280.0bcB	334.3cB	6.8aA	70.4aA
Cutting	253.6abC	288.3bcC	2.0aA	60.8aB
P fertilization	292.8cD	152.8abB	6.2aA	223.2bC
Control	220.8aC	192.0aC	7.2aA	96.8aB

Means of each treatment within the same growing season followed by the same small letter and between years followed by the same capitals are not significantly different at $p \leq 0.05$

As far as the individual treatments are concerned, the results were the following. Burning resulted in significantly lower density values than the control in the autumn of the first growing season but it recovered in spring. The same pattern was repeated in the second and fourth growing seasons. In contrast, no significant differences were caused in the autumn of the third season while in the spring the treatment produced significantly higher density than the control (Tables 1 and 2).

Irrigation did not produce significant different legume densities compared with the control in both seasons of the four growing seasons except in the spring of the second year when significantly higher density was recorded compared with the control. Similar were the results obtained in the treatment of cutting as well (Tables 1 and 2).

Digging did not significantly affect legume density in the autumn of the first growing season, but the density obtained in the spring was significantly higher than the control. In the second growing season, however, it significantly reduced density during autumn but in the spring produced significantly higher density than the control. The significant reduction appeared again in the autumn of the forth season without recovery during spring. P fertilization finally produced inconsistent results in the first three growing seasons but significantly higher densities than the control and all the other treatments in both seasons of the fourth year.

Similar results with the total legume density were also found with the density of the individual legume species. More specifically, there was a sharp reduction of their density between the second and third growing season and an impressive recovery in the

forth which, however, did not reach the levels of the first. In *O. aequindentata*, a dominant legume species, its density was reduced by 95% on the average for all treatments between 1995 and 1996 and recovered by 175% between 1996 and 1997. Among the treatments, however, only burning and digging resulted in significantly higher than the control densities in 1996, an effect that was also maintained in 1997 but only for the latter treatment. All the other treatments produced no significant results (Table 3).

Among *Trifolium* spp., *T. scabrum*, the second dominant legume, did not appear at all in 1996 while the density in 1997 was on the average much less than the levels of 1995. Among the treatments, only P fertilization produced significantly higher density than the control in 1997. The other treatments produced no significant results in any of the two years. *T. angustifolium*, a third species in terms of dominance, did not appear at all in 1996, except in the irrigation treatment and the control, while it recovered only in the digging treatment. In *T. campestre*, its density was decreased by 95% on the average between 1995 and 1996 and increased by 92% between 1996 and 1997. Among treatments, only P fertilization produced significant results compared with the control in 1997 (Table 3).

Hippocrepis multisiliquosa did not also appear in 1996, except in the irrigation treatment and the control, while it recovered only in the burning, digging and cutting treatments as well as in the control (Table 3).

Among *Medicago* spp., finally, overall density was decreased by 88% between 1995 and 1996 in *M. minima* and almost by 100% in *M. disciformis* while it increased by 93% and almost by 100% between 1996 and 1997 in the two species, respectively. They were the only species where 1997 density levels exceeded those of 1995. The applied treatments though affected only *M. minima*, which was more dominant than *M. disciformis*. Specifically, burning, irrigation and P fertilization reduced significantly its density compared with the control, while the latter treatment seemed to have positively affected it in the fourth season (Table 3).

Biomass

Like density, legume biomass changed both among growing seasons and among treatments. More

Table 3 Mean spring density (plants/m^2) of the dominant legume species in the various treatments for three years

Treatments	Onobrychis aequindentata			Trifolium scabrum			Trifolium angustifolium			Trifolium campestre			Hippocrepis multisiliquosa			Medicago minima			Medicago disciformis		
	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997
Burning	91.6a	15.0c	10.4b	78.5a	0.0	20.0a	16.0a	0.0a	0.0a	37.5a	0.4a	1.6a	3.2a	0.0a	8.0b	4.4a	0.4a	4.0a	2.4a	0.4a	8.8a
Irrigation	77.6a	1.4a	6.4ab	54.5a	0.0	24.0a	11.3a	0.2a	0.0a	23.5a	1.2a	4.8a	40.0b	0.2a	0.0a	4.2a	0.2a	6.4a	2.2a	0.2a	4.0a
Ploughing	88.0a	5.8b	23.2c	44.1a	0.0	27.2a	7.0a	0.0a	5.6b	10.3a	0.2a	7.2a	44.0b	0.0a	11.2b	11.4b	0.0a	3.2a	7.4b	0.4a	0.8a
Cutting	73.6a	0.6a	1.6a	101.0a	0.0	33.6a	10.5a	0.0a	0.0a	24.8a	0.2a	2.4a	0.0a	0.0a	0.8a	11.0b	0.6a	7.2a	2.6a	0.6a	3.2a
P Fertilisztion	86.8a	2.6ab	12.8b	53.8a	0.0	92.4b	2.5a	0.0a	0.0a	20.3a	1.0a	49.6b	52.0b	0.0a	0.0a	4.8a	0.6a	56.0b	4.4ab	0.2a	8.8a
Control	78.4a	1.2a	8.8ab	59.8a	0.0	52.8a	17.5a	0.6a	0.0a	17.0a	0.4a	8.0a	12.8ab	0.6a	1.6ab	10.4b	1.6a	9.6a	3.6ab	0.8a	7.2a

Means of each treatment within the same year followed by the same letter are not significantly different at $p \leq 0.05$

Table 4 Means of total legume biomass (g/m^2) in the spring in the various treatments for four years

Treatments	1994	1995	1996	1997
Burning	66.42aB	82.59cdB	7.46bA	18.54abA
Irrigation	106.53abC	39.97abB	0.34aA	7.33aA
Digging	109.61abB	114.62 dB	1.43aA	36.13abA
Cutting	108.50abB	18.96aA	0.36aA	7.57aA
P fertilization	123.82bC	59.99abcB	2.45aA	121.72cC
Control	130.27bC	64.91bcB	4.08aA	47.48bB

Means of each treatment within the same year followed by the same small letter and between years followed by the same capitals are not significantly different at $p \leq 0.05$

specifically, it was significantly reduced in four treatments (except burning and digging) between 1994 and 1995, but in all of them between 1995 and 1996. However, no changes occurred in the four of them between 1996 and 1997, when only P fertilization and the control treatments resulted in significantly higher biomass (Table 4).

As far as the individual treatments are concerned, burning significantly reduced legume biomass compared with the control in the first year but it recovered the second year, while in the third the treatment produced significantly higher biomass than the control to return again to control level during the fourth year. Irrigation did not produce significant results except in the fourth year when the biomass produced was significantly lower than the control. Digging did not produce any significant results at all. Cutting resulted in significantly lower biomass in the second and third years, while P fertilization in significantly higher biomass only in the last year of the experiment. The results suggest, as in the case of density, that the treatments applied had no effect (digging), or temporary effects (burning, irrigation and cutting) or a delayed positive effect (P fertilization) on legume biomass (Table 4).

For the individual species, their biomass reacted the same way as the total biomass; they were greater and persistence differences among years than among treatments. *Onobrychis aequinetata*, for example, reduced its biomass on the average from the 1995 to 1996 and it increased it from the 1996 to 1997. Among treatments, only burning resulted in increased biomass compared with the control in 1996; cutting resulted in reduced biomass compared with the control in 1995.

Table 5 Mean biomass (g/m^2) of the dominant legume species in the various treatments for three years

Treatments	Onobrychis aequindentata			Trifolium scabrum			Trifolium angustifolium			Trifolium campestre			Hippocrepis multisiliquosa			Medicago minima			Medicago disciformis		
	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997	1995	1996	1997
Burning	41.66ab	7.35ab	10.87ab	9.14b	0.00	1.95a	3.96a	0.00a	0.00a	2.07a	0.00a	0.05a	0.34a	0.00	1.48a	12.37a	0.07a	0.34a	1.71a	0.04a	0.83a
Irrigation	30.50ab	0.21a	3.97ab	3.45a	0.00	2.11a	1.18a	0.01a	0.01a	0.65a	0.00a	0.22a	2.02a	0.00	0.00a	1.86a	0.04a	0.56a	0.74a	0.02a	0.35a
Ploughing	59.26b	1.19a	24.40c	3.21a	0.00	9.56a	0.60a	0.00a	0.41b	0.30a	0.00a	0.92a	15.03c	0.00	1.39a	4.42a	0.00a	0.42a	6.68b	0.01a	0.06a
Cutting	11.32a	0.32a	0.19a	4.26a	0.00	4.72a	0.22a	0.00a	0.00a	0.63a	0.00a	0.12a	0.00a	0.00	1.18a	1.45a	0.02a	0.77a	0.56a	0.03a	0.62a
P fertilization	42.44ab	0.68a	12.60b	4.54ab	0.00	33.56b	0.11a	0.00a	0.00a	2.01a	0.00a	10.88b	0.26a	0.00	0.00a	1.02a	0.01a	52.06b	0.70a	0.01a	6.82b
Control	50.70b	0.26a	7.58ab	6.24ab	0.00	6.08a	1.40a	0.01a	0.00a	0.94a	0.00a	0.53a	3.55b	0.00	0.18a	1.86a	0.21a	1.46a	0.77a	0.10a	0.88a

Means of each treatment within the same year followed by the same letter are not significantly different at $p \leq 0.05$

All the other treatments produced no significant results (Table 5).

Among *Trifolium* spp., *T. scabrum* did not produce any biomass at all in 1996 like density but it recovered completely in 1997. Only P fertilization produced significant higher biomass than the control in 1997, while irrigation, digging and cutting produced significant lower biomass than the control in 1995. *T. angustifolium* did not produce also biomass in 1996 except in the irrigation treatment while in the other two years the biomass produced was negligible. In this year, digging resulted in increased biomass compared with the control in 1997. *T. campestre* behaved also the same way as *T. scabrum* (Table 5).

Hippocrepis multisiliquosa did not produce also measurable biomass in 1996 and the applied treatments affected it during the second year (1995). More specifically, burning, irrigation, cutting and P fertilization resulted in significantly lower biomass while digging in significantly higher biomass than the control (Table 5).

Among *Medicago* spp., finally, *M. minima* had almost null biomass in 1996 but recovered completely in 1997, especially the treatment with P fertilization which produced significantly higher quantity than the control. Similar behaviour was displayed by *M. disciformis* too, which in addition was favoured by the digging treatment in the second season (1995).

Discussion

The great reduction of legume density in the autumn of the third growing season should be attributed to the adverse weather conditions of the months October and November when legumes mainly germinate and get established. According to George et al. (1984), germination occurs when rainfall is at least 25 mm, which is much more than the 2.6 mm that fell in October of that particular season. In November, although rainfall was sufficient (44 mm), the low temperatures (below normal) apparently prevented the mass germination of legumes. The fact that legume density was increased in the spring suggests that leguminous seeds continued to germinate after November but in limited numbers since this particular season was quite low compared to the other seasons. This late germination (after November)

should explain the increase of the spring density as compared with the autumn one in the other growing seasons, too. The overall decrease though of the spring density in the second growing season compared with the autumn one, on the contrary, should be attributed to the relatively low temperatures of the winter months, especially December and January. This means that moisture and temperature in October and November and temperatures in November and December are the critical factors for legume germination and establishment, while too low temperatures in the winter months may kill the established seedlings. Special research should be carried out to verify all these interactions between weather parameters and legume seed germination.

The recovery of legumes during the fourth growing season, after the great reduction of the third one, should be attributed to the seed reserves in the seed bank. The fact that the seeds of annual legumes are part of the permanent seed bank in the mediterranean environment has been confirmed by several investigators (Rice 1989; Cocks and Osman 1996; Ehrman and Cocks 1996; Cocks 1992a, b; Del Pozo and Aronson 2000; Sulas et al. 2000) and suggests their high adaptation and resilience to mediterranean climate. However, not all the species were equally affected by the adverse weather conditions of the third year or recovered in the fourth growing season. For example, *O. aequindentata* was the least while *T. scabrum* the most affected species, but the latter recovered more impressively than the former. An impressive recovery was also displayed by annual medics, especially *M. minima*, while *T. campestre* recovered much more easily than *T. angustifolium*. These results show that individual annual legumes have different sensitivities to extreme weather conditions and different capabilities to recover after them.

Among the factors studied, P fertilization was the most effective in improving both legume density and biomass and helping most species recover from the shock of the third growing season, especially *T. scabrum*, *T. campestre* and *M. minima*. The reduced density and biomass in the second growing season should be attributed to the limited rainfall that fell during this season (only 74% of the long term average). The results of this factor were expected. Several authors have found that P fertilization favours the establishment and growth of legumes in

mediterranean grasslands at the expense of grasses (Papanastasis and Koukoulakis 1988; Menke 1989; Osman et al. 1991; Henkin et al. 1996; Henkin and Seligman 2000; Papanastasis and Papachristou 2000; Rochon et al. 2004).

Burning is known to favour legumes in two ways: by breaking their seed dormancy and removing the competitive vegetation. Several investigators have found an increased legume density in mediterranean ecosystems, especially in forests after fire (Papavasiliou and Arianoutsou 1993; Kazanis and Arianoutsou 1996; Arianoutsou and Thanos 1996). The negative effects that burning had in this study, especially in the autumn densities, although they did not persist, could be attributed to a possible partial destruction of the surface seeds by fire which produced a great part of the autumn seedlings. Among these, *O. aequindentata* was the most and *M. minima* the least favoured species.

The lack of any appreciable effect of autumn irrigation could be attributed to the fact that it was applied in November and not in October which turned out to be the most critical month for seed germination. As for March irrigation, apparently it was not needed except in the second growing season which had a dry spring.

Although digging was a quite "severe" treatment, legumes are adapted to such a factor, since density could recover by the end of the growing season. This recovery can be attributed to the modification of the permanent seed bank as seeds from the deeper soil layer could come to the surface and germinate. The treatment especially favoured *O. aequindentata*, *T. angustifolium*, *H. multisiliquosa* and *M. disciformis*.

Several researchers claim that cutting favours legumes since it removes competitive vegetation, especially grasses (Naveh and Whittaker 1979; Sternberg et al. 2000). On the other hand, cutting during the bloom stage may limit the capacity of legumes to produce seeds and subsequently persist (Briske 1989; Tsiouvaras et al. 1993). The lack of any appreciable effects of this factor in the present study can be attributed to the large variety of species present which do not bloom at the same time suggesting that the one cut applied in spring was not enough to impose significant results, especially on legume density. Legume biomass, however, was negatively affected in the second growing season, which was relatively dry suggesting a negative

interaction between cutting and drought. The species involved in this interaction were *O. aequindentata*, *T. scabrum* and *H. multisiliquosa.*

Conclusion

It can be concluded that annual legumes are vulnerable both to weather changes and to management interactions. Nevertheless, they display a remarkable capacity to overcome these changes and interventions due to their opportunistic behaviour. This behaviour is dictated by the different strategies of the component species which allows them to survive in a variable and highly unpredictable natural environment. On the other hand, annual legumes constitute an important asset of mediterranean grasslands. The high biomass recorded in this study, although reduced over the four years, indicates their importance both for ecosystem functioning through their capacity to fix atmospheric nitrogen and for feeding grazing animals especially sheep, due to their high nutritional value.

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