Use of water-stress tolerant *Lotus creticus* and *Cynodon dactylon* in soil revegetation on different slopes in a Mediterranean climate

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The performance of the legume *Lotus creticus* growing in mixture with the grass *Cynodon dactylon* was compared with a *L. creticus* pure stand in field conditions on 0°, 19° and 32° slopes in Barcelona, Spain. Two harvests were carried out, the first in early summer and the second in mid-autumn. From winter to spring, *Lotus creticus* growing in mixture contributed a greater biomass than the grass. Total shoot biomass of pure stand and mixture differed among slopes. On the same slope shoot biomass in the mixture and pure stands was not significantly different. The vegetation cover of the mixture was approximately 100%, 90% and 86% on the 19°, 0° and 32° slopes, respectively. The vegetation cover in the pure stand on the three former slopes was approximately 100%. During summer the water deficit was important on the 32° slope and *L. creticus* plants mortality in the mixture and pure stands was recorded. In autumn, legume biomass in mixture was lower than grass. The vegetation cover in mixture and pure stands was nearly 100% on the 0° and 19° slopes, and 60% on the 32° slope. During the experiment changes in photosynthesis, water use efficiency and water potential in both species were recorded. *Lotus creticus* plants growing in mixture were not affected in their physiological variables as compared with those in the pure stand. On a same slope, the mixture and pure stands did not differ in total shoot biomass. In mixture the lowest biomass production in one species was compensated by an increase in the other.

Key words: *Cynodon dactylon*, digital image analysis, ecology, *Lotus creticus*, revegetation
Introduction


Desertification in the Mediterranean region is mainly due to vegetation cover reduction and soil erosion (Kosmas et al. 2000). Mediterranean climate is characterized by hot, dry summers and cool, cold winters, which limits the use of different species for soil revegetation (Mooney et al. 1974, Savé et al. 1999). Therefore, the use of native species is recommended due to their tolerance to water stress (de Herralde et al. 1998, Sánchez-Blanco et al. 1998, Morales et al. 2000, Franco et al. 2001).


Mixtures of grass and legume are recommended over pure stands for soil revegetation mainly due to five reasons: (1) nitrogen transfer from legume to associated grass (Mallarino et al. 1990, Gebhart et al. 1993, Farnham & George 1994); (2) high biomass production under variable environmental conditions (Jolliffe 1997); (3) resource use efficiency (Jolliffe 1997, Naidu & Harwood 1997, Piper 1998); (4) soil fertility improvement as well as moisture retention capacity (Gebhart et al. 1993, Choi & Wali 1995); (5) increase of organic matter in the soil (Choi & Wali 1995).

Ecophysiological responses and biomass production of *L. creticus* and *C. dactylon* growing together and in pure stands in greenhouse under water stress were analyzed by Vignolio et al. (2002). Both species showed qualities that make them good candidates for soil revegetation. However, their performance in field conditions remains unknown.

In this work the performance of a *L. creticus* pure stand and a mixture of *L. creticus* and *C. dactylon* were compared. The work was carried out in field conditions on soils with 0°, 19° and 32° slopes. Vegetation cover, shoot and root biomass as well as the physiological responses of the plants were recorded.

Material and methods

Study site

The study site is located at Recerca i Tecnologia Agroalimentàries Institut (IRTA), 41°25′N and 2°21′E, at 85 m above sea level in Cabrils, Barcelona, Spain. The work was conducted from December 1998 to October 1999. Mean (± S.E.) maximum and minimum temperatures are 13.59 ± 0.18 °C and 5.55 ± 0.19 °C in winter and 26.00 ± 0.22 °C and 18.28 ± 0.21 °C in summer. Annual mean precipitation is 592 ± 38.52 mm. All average data comprise the period between

Table 1. Physical and chemical properties of soil defined according to the USDA system.

<table>
<thead>
<tr>
<th>Soil characteristic</th>
<th>Means ± 1 S.E. (n = 4)</th>
</tr>
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<tbody>
<tr>
<td>pH (1:2.5)</td>
<td>7.12±0.34</td>
</tr>
<tr>
<td>C.E. (25 °C, 1:5; ds/m)</td>
<td>0.082±0.003</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>1.80±0.51</td>
</tr>
<tr>
<td>P (NaHCO₃ 0.5M; ppm)</td>
<td>31.00±3.76</td>
</tr>
<tr>
<td>N (Kjeldahl; %)</td>
<td>0.075±0.013</td>
</tr>
<tr>
<td>K (NH₄AcO 1N; ppm)</td>
<td>85.25±8.43</td>
</tr>
<tr>
<td>Sand (0.5 &lt; to &lt; 2 mm; %)</td>
<td>24.67±4.51</td>
</tr>
<tr>
<td>Sand (0.05 &lt; to ≤ 0.5 mm; %)</td>
<td>51.55±1.87</td>
</tr>
<tr>
<td>Sand (0.05 &lt; to 2 mm; %)</td>
<td>76.22±3.34</td>
</tr>
<tr>
<td>Silt (0.002 &lt; to ≤ 0.05 mm; %)</td>
<td>14.50±2.11</td>
</tr>
<tr>
<td>Clay (&lt; 0.002 mm; %)</td>
<td>9.27±1.29</td>
</tr>
</tbody>
</table>
Soil revegetation on different slopes in a Mediterranean climate

1981 and 1998, and they were provided by IRTA weather station.

Plant species

Lotus creticus and C. dactylon specimens were provided by IRTA. On 11 December 1998, L. creticus with a primary (5 cm long) and two secondary stems were planted in pots (4 × 4 × 5 cm depth) filled with peat and perlite (2:1 v/v, pH: 6.6). On 20 January 1999, C. dactylon was cultivated from buds and uniform-sized rhizomes. Lotus creticus stems require more time to develop roots, which is why they were cultivated earlier than C. dactylon. Both species were cultivated in greenhouse under well-watered conditions.

Experimental design, soil and plant transplanting

The experiment consisted of (1) L. creticus pure stand, and (2) L. creticus and C. dactylon growing in mixture. A sandy soil with 0°, 19° and 32° slopes was used (USDA system, Soil Survey Staff 1975, Table 1). The orientation of the 19° and 32° slopes was with an azimuth of 38° S–SE (Marfà 1990). On each slope six plots (three repetitions × two species combinations) of 90 × 160 cm (downward) were established and they were separated by a 15-cm wide plant border (Fig. 1).

Irrigation and climatic conditions

Water volume used for irrigation for each experimental month was estimated from rainfall data between 1980 and 1998. Dripping irrigation (0.326 l m⁻² min⁻¹) without soil erosion was applied from 11 February until 16 June (126 days after transplant, DAT). Irrigation was done every two days, using up the monthly estimate of water. Data on temperature, relative air humid-

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Fig. 1.— A: Schematic illustration of experimental plots, Lotus creticus (X) and Cynodon dactylon (O) growing on the slopes. References: Subplot plants are located inside the internal rectangles (60 × 40 cm); The arrow indicates the plant row where water potential was measured; Head arrow indicates the plastic band for dripping irrigation; Soil water content was measured on border plants. — B: Arrangement of higher (H) and lower (L) subplot on the slopes.

Fig. 2.— Mean maximum (Max. T.) and minimum temperatures (Min. T.) and relative air humidity (R.H.) during the experimental period.
ity (Fig. 2), precipitation (Fig. 3A) and incident radiation on the 0° slope were provided by IRTA. Incident radiation on the 0° slope was used to estimate the incident radiation on the other two slopes (Duffie & Beckman 1980, Marfà 1990). The data were verified with a Sunfleck Ceptometer (Decagan Devices, Inc. Model SF-80) as well as with information from literature (Mitjá & Batalla 1982).

Soil water content

Soil water content was measured to a depth of 16 cm with a time-domain reflectometry (TDR, Soil Moisture Meter, Trime-FM IMKO, Germany) and a 16 cm long probe (P2G). Five downward-measurements on each slope were recorded (Fig. 1A) in winter (18 February, 8 and 17 March), in spring (31 March, 16 and 30 April, 13 May, 9 June), in summer (23 June, 22 July, 16 and 25 August) and in autumn (22 September, 9, 14 and 20 October). Water content was recorded for the upper 16 cm of soil since *C. dactylon* has 58% of its root biomass (average of 10 genotypes) at this depth (Hays *et al.* 1991). There is no information available of the root biomass distribution of *L. creticus*. Difference in soil water contents were tested with ANOVA followed by LSD test.

Plant mortality

Plant mortality was regularly recorded in each subplot. A plant was considered dead when the shoot was dry and without a sprout. Dead plant percentage was recorded and averaged in the subplots. The plants that died during the first 7 DAT were not included in the records.

Biomass harvest

Shoot biomass

Two harvests of shoot biomass were carried out, the first on 121 DAT (11 June) and the second on 255 DAT (23 October) over the subplots of each plot (Fig. 1). Both harvests were done when some of the species combinations achieved approximately an 100% cover. The plants were hand-clipped and the dry weight was recorded (72 h, 60 °C). The remaining plant cover in each subplot was approximately 7%. The shoot biomass of the borders was harvested and eliminated. Biomass per plant of *L. creticus* was estimated as the biomass harvested in each subplot and divided by the number of living plants. For each plot, the records of each subplot were averaged.

Root biomass

Root biomass was harvested on 255 DAT (23 October) after the second shoot biomass harvest. Root samples were taken out from the upper 50 cm soil layer. Five cores (8 cm diameter by 10 cm depth) were taken from the high subplot (Fig. 1A). Root harvesting in the pure stand was done between two plants of the same species, and between individuals of different species in the mixture (Fig. 1A). Roots were washed and the dry weight was recorded. In mixture, root biomass was not identified to species.

Differences in shoot and root biomasses were tested with ANOVA followed by LSD test.
ferences in shoot biomass per plant of *Lotus creticus* growing in the mixture and pure stands were analysed with Student’s *t*-test.

### Vegetation cover

Vegetation cover was recorded on photographs analyzed with a digital image analyzer (Delta T. Image Analysis System, England). Colour photographs (10 × 15 cm) of each subplot were taken vertically from a height of 1 m, using a detachable elevated board. A transparent slide was put on each photograph and the shoot biomass was coloured black. Next, having the subplot surface as a reference, vegetation cover was recorded on the slide. Subplot records were averaged for each plot. A comparable method was used by Hansjörg and Thomas (1996), and they found a high determination coefficient (*R*²) of 0.96 to 0.99 between the total leaf area, determined with the leaf-area meter, and species cover determined from the image analysis.

### Physiological response of plants

Photosynthesis and transpiration rates were recorded simultaneously with an ADC LCA2 (Analytical Development, UK) in full sunlight at midday, in spring (4 April and 3 June), in summer (22 July and 24 August) and in autumn (20 September and 21 October).

Determinations on the 19° and 32° slopes were carried out in the high subplot (Fig. 1). Instantaneous water use efficiency (WUE) was calculated by dividing photosynthetic rate with transpiration rate, both of which were measured under identical conditions.

Water potential was measured with a pressure chamber Soilmoisture 3005 (Soilmoisture Equipment, USA) in the morning (05:30 solar hrs) and midday (12:00 solar hrs), in summer (9 and 26 July, 2 August, 2 and 21 September) and in autumn (9 and 20 October). Measurements at *L. creticus* stems and *C. dactylon* stolons were made (Fig. 1). In each species combination, three repetitions were recorded.

Photosynthesis, WUE and water potential were tested by ANOVA. Mean comparison was made with LSD test. Physiological variables of *L. creticus* growing in mixture and as pure stand on the same slope were compared with Student’s *t*-test.

### Experiment maintenance

*Cynodon dactylon* grew to the outside of the experimental subplots. No rhizomes were recorded in *L. creticus* pure stand during root harvest. This would indicate that underground grass interference with *L. creticus* in pure stand could be negligible. Plots were kept weed-free by hand. No insects or other animals were observed during the whole experiment.

### Results

#### Radiation

The total incident radiation on the 0°, 19° and 32° slopes was 170 860, 179 264 and 176 983 kJ m⁻², respectively. At the first shoot biomass harvest, the total incident radiation on the 19° and 32° slopes was approximately 7.40% higher than on the 0° slope. Between the first and second shoot biomass harvests, the total incident radiation on the 19° slope was approximately 3% higher than on the 0° and 32° slopes.

#### Soil water content and climatic conditions

From February to April, (winter–early spring) the evapotranspiration (ETP) increased and the scarce rainfall was compensated with irrigation (Fig. 3A). During summer (June–September) the rainfall was also scarce and ETP was high (Fig. 3A). Between September and October rainfall increased and ETP diminished (Fig. 3A). Soil water content differed among the slopes (*p* < 0.048) and the seasons (*p* < 0.0001) (Fig. 3B), being the lowest on the 32° slope in summer (Fig. 3B). During the experimental period the rainfall was 385 mm and only on 16 September it was 105 mm, or 27.27% of the total (Fig. 3A).
Mortality of plant species was recorded on the different slopes. The plant mortality (mean ± S.E.) was 16.66% ± 8.33% and 8.33% ± 4.16%, respectively. The plant mortality in the mixture on the 0°, 19° and 32° slopes was 33.33% ± 20.04%, 16.66% ± 8.33% and 25.00% ± 14.43%, respectively.

Biomass harvest

Shoot biomass

Total shoot biomass in the mixture at the first and second harvests differed only among slopes, $p < 0.015$ and $p < 0.033$, respectively (Fig. 4). In the mixture and pure stands, the lowest biomass was recorded on the 32° slope (Fig. 4). At the first harvest, $L. creticus$ in mixture contributed 30.33%, 45.71% and 20.27% of the total biomass on the 0°, 19° and 32° slopes, respectively (Fig. 4A). At the second harvest, $L. creticus$ in mixture contributed 30.33%, 45.71% and 20.27% of the total biomass on the 0°, 19° and 32° slopes, respectively (Fig. 4B).

At the first harvest, shoot biomass per plant of $L. creticus$ growing in the different experimental conditions was not significantly different, average 17.35 ± 0.95 g plant$^{-1}$. At the second harvest, shoot biomass per plant of legume was not significantly different between the pure and mixture stands, though it was lower on the 32° slope (5.52 ± 1.09 g plant$^{-1}$) than on the 0° (15.15 ± 1.30 g plant$^{-1}$) and 19° (20.49 ± 3.24 g plant$^{-1}$) slopes.

Root biomass

Total root biomass did not significantly differ among slopes and species combination, and the average was 1.40 ± 0.27 g (in 2513.28 cm$^3$ of soil). About 60% of the root biomass accumulated in the mixture was found in the upper 20 cm soil layer, and the same root biomass percentage in the pure stand was found between 0–40 cm depth in the soil (Fig. 5).
Vegetation cover

During the first months, the pure stand had more vegetation cover than the mixture. For example on 30 April (79 DAT), the vegetation cover in the mixture was 25.99% ± 2.09% (Fig. 6A) and in the pure stand it was 42.35% ± 2.49% (Fig. 6B). At the first harvest of shoot biomass, in the pure stand and only the mixture growing on the 19° slope the vegetation cover was 100% (Fig. 6). After the first harvest, both species sprouted; however, a reduction in vegetation cover was recorded between July and August (summer). Between September and October the vegetation cover increased and when the second harvest was done, it was approximately 100% on the 0° and 19° slopes and 60% on the 32° slope (Fig. 6).

Photosynthesis and WUE

Photosynthesis and WUE of both species presented comparable patterns on the three slopes during the experimental period (Figs. 7 and 8). Physiological measurements in C. dactylon were not recorded in March since the plants had a great percentage of dry leaves, attributed to low temperatures. Photosynthesis and WUE of L. creticus growing pure and in mixture were not significantly different and only differed among the seasons (p < 0.0001) (Figs. 7 and 8). Photosynthesis and WUE of C. dactylon differed among the seasons (p < 0.0001) and slopes (p < 0.005). In both species, season × slope interaction was recorded for WUE (p < 0.032).

![Fig. 6. Mean (± S.E.) vegetation cover on the 0°, 19° and 32° slopes. — A: Lotus creticus and Cynodon dactylon mixture. — B: Lotus creticus pure stand. Arrow and head arrow indicate dates of first and second shoot biomass harvest, respectively; DAT = days after transplanting.](image)

![Fig. 7. Mean photosynthesis recorded on the 0°, 19° and 32° slopes. — A: Lotus creticus pure stand. — B: Lotus creticus in mixture with Cynodon dactylon. — C: Cynodon dactylon in mixture with Lotus creticus. Bars with different letters indicate significant differences (p < 0.05) among photosynthesis rate.](image)
Water potential

Water potential of *L. creticus* in mixture and as pure stand did not significantly differ. In both species the water potential differed among seasons and time of day (*p* < 0.0001), the lowest values being recorded at mid-day and in summer (Table 2). Differences among slopes in *C. dactylon* were recorded (*p* < 0.003), those being significantly lower on the 32° (–2.20 ± –0.33 MPa) than on the 0° (–1.21 ± –0.22 MPa) and 19° (–1.26 ± –0.24 MPa) slopes.

### Discussion

In this region where the rainfalls are short but intense, soil erosion increases if soils lack vegetation cover. It is therefore of utmost importance which species are used to protect the soil from natural as well as anthropogenic degradation.

Until the first shoot harvest, the 19° and 32° slopes received the same incident radiation and water amount. However, the lowest shoot biomass was recorded in the mixture on the 32° slope due to weak *C. dactylon* growth, which was attributed to low winter temperature (Fernández 2002) and short day length (Burton *et al.* 1988) at the beginning of the experiment (Fig. 2). In the mixture at the second harvest of shoot biomass, the lower *L. creticus* biomass relative to *C. dactylon* was due to legume mortality. However, plant mortality would not totally explain the differences in the vegetation cover recorded at the end of the experiment (Fig. 6). For example, the plant mortality and cover of *L. creticus* growing in the pure stand on the 32° slope were 8.33% and 53.65%, respectively. On the other hand, on the 0° slope the plant mortality in the pure stand was 16.66% but the cover was approximately 100% (Fig. 6). These results showed that, besides plant mortality, other factors, such as soil water content, affected plant growth and vegetation cover (Fig. 3). *Lotus creticus* mortality was not recorded in greenhouse

<table>
<thead>
<tr>
<th>Table 2. Mean (± S.E.) <em>Lotus creticus</em> and <em>Cynodon dactylon</em> water potential (MPa) in different seasons and time of day. <em>Lotus creticus</em> water potential was average between mixture and pure stand. Different letters indicate significant differences (<em>p</em> &lt; 0.05) between season or time.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
</tr>
<tr>
<td><em>L. creticus</em></td>
</tr>
<tr>
<td><em>C. dactylon</em></td>
</tr>
<tr>
<td><strong>Time of day</strong></td>
</tr>
<tr>
<td><strong>Species</strong></td>
</tr>
<tr>
<td><em>L. creticus</em></td>
</tr>
<tr>
<td><em>C. dactylon</em></td>
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conditions when both species were growing in the mixture and pure stands under water deficit (Vignolio et al. 2002). These differences may be, among other factors, due to species-specific responses to climatic conditions after shoot harvest. *Cynodon dactylon* reproduces vegetatively by rhizomes and stolons; therefore, plant mortality may be lower than in *L. creticus*, which has to regrow from crown or basal stem buds.

In this experiment the soil had a low water retention capacity due to the small amounts of organic matter and high sand content. Water deficit becomes a more important factor as the slope increases (Carroll et al. 2000, Loch et al. 2000). In this experiment the soil water amounts differed among the slopes, being lower on the 32° slope (Fig. 3). Low water potential recorded in water stress may be overcome by osmotic adjustment and plastic changes in plant roots (Kramer 1983, Savé et al. 1993, 1996). Under water deficit, osmotic adjustment in *L. creticus* (Savé et al. 1996, Sánchez-Blanco et al. 1998) and *C. dactylon* (Utrillas & Alegre 1997) was recorded. In the present study, the daily osmotic potential changes in both species were recorded together with the water potential (data not presented). In *L. creticus*, the maximum and minimum osmotic potential was between −1.499 to −4.414 MPa and in *C. dactylon* between −1.739 to −4.768 MPa. Our results are in agreement with those reported by Utrillas et al. (1995, cited by Utrillas & Alegre 1997) for *C. dactylon* water stress tolerance growing in field conditions during a dry summer. Under water deficit, a specific length and root area increase was recorded in both species growing in the mixture and pure stand (Vignolio et al. 2002). These changes can increase water and nutrient absorption and ameliorate or avoid consequences of water deficit (Hoogenboom et al. 1987, Duncan & Carrow 1999). We did not record root biomass separately for *C. dactylon* and *L. creticus*. Root biomass in the mixture was found to be shallower than in the pure stand of *L. creticus*. *Cynodon dactylon* concentrates 40% or more of the root biomass and rhizomes in the uppermost 20–25 cm of soil (Hays et al. 1991, Huang et al. 1997, Fernández et al. 2002). Due to this fact and others mentioned in different works on water stress tolerance, *C. dactylon* is an important grass for soil revegetation.

Photosynthesis in both species differed among seasons due to changes in soil water content, temperature and radiation. Photosynthesis in *L. creticus* increased between March and April and was accompanied by an increase of air temperature and radiation (Vignolio et al. 2002). The maximum photosynthesis values were first recorded in *L. creticus* on 20 April and later in *C. dactylon* on 1 June, this being consistent with the different optimal temperatures of *C.* and *C.* species growing under water deficit (Teare et al. 1973). However, our results agree with those obtained for both species growing in mixture, pure stand and under water stress (Vignolio et al. 2002). Although *C. dactylon* presented a higher photosynthetic rate than *L. creticus* at a certain stage of the experiment (Fig. 7), also the transpiration rate of the grass was higher (the data were not presented); therefore, its WUE was not higher than the WUE in the legume (Fig. 8) (Vignolio et al. 2002).

The physiological measurements and biomass production of *L. creticus* growing in mixture with *C. dactylon* were not different from those observed in the pure stand. Although on the same slope the total shoot biomass in the mixture and pure stands was not significantly different, both species were differently affected by experimental conditions. In mixture a lower biomass production of a species was compensated by an increase in the other. Soil revegetation can be considered as a succession process where some species are replaced by others. Ecological changes of these processes will depend, for example, on resource use efficiency, seed bank, biological interaction (competition, plant-animal interaction, allelopathy), weather fluctuations, reproductive strategies, type, intensity and frequency of disturbance (Wali 1999). Although *L. creticus* and *C. dactylon* clearly are potential candidates for soil revegetation, some observations mentioned above can affect the species in mixture and further studies must be carried out on the two species.

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References


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