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Self-reseeding annual legumes for cover cropping in rainfed managed olive orchards

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Abstract

Given the environmental impact of nitrogen (N)-fertilizer manufacture and use, the sustainable management of agro-systems should be sought by growing N-fixing legumes. In this work, eleven self-reseeding annual legumes were grown in pure stands as mulching cover crops in a rainfed olive orchard managed without grazing animals. Dry matter yield, N content in above-ground biomass, groundcover percentage and persistence of the sown species were assessed during four growing seasons. All covers provided enough soil protection over the year, with living plants during the autumn/winter period and a mulch of dead residues during the summer. The legumes overcame a false break observed in the third year recovering the dominance of the covers in the fourth growing season. This means that the seed bank established in previous seasons ensured the persistence of the sown legume even when a gap in seed production occurred. The early-maturing cultivars produced less biomass and fixed less N (approx. 50 kg N/ha/yr present in the above-ground biomass) than the late-maturing ones, but would compete less for water since the growing cycle finished earlier in the spring. They seem best suited to being grown in dry farmed olive orchards with low N demand in drought prone regions.

Additional key words: pasture legumes; mulching; *Olea europaea*; nitrogen management; groundcover percentage; persistence.

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Introduction

Cover cropping in perennial tree crops and vineyards has been increasing in the last decades, particularly in orchards of temperate climates without problems of water shortage and also in irrigated plantations of drier regions. The great advantages of cover cropping are the efficient reduction in soil erosion (Martínez *et al.*, 2006; Gómez *et al.*, 2009) and the increase of soil organic matter (Montanaro *et al.*, 2010; Ferreira *et al.*, 2013). Cover cropping may also improve many other physical, chemical and biological properties of soil compared to frequent tillage (Moreno *et al.*, 2009; Ramos *et al.*, 2010). Cover cropping is frequently used in combination with herbicides. A common groundcover management system in orchards is sod alleys and herbicide-maintained tree rows (Sirrine *et al.*, 2008).

In the olive orchards of the Mediterranean basin, however, tillage and herbicides are still currently the dominant weed control practices due to their effectiveness in spite of their link to major environmental issues. In drought-prone regions, cover cropping in orchards and vineyards is progressing slowly. Cover crops compete for resources, particularly for water, which may reduce crop production (Rodrigues *et al.*, 2011a; Gucci *et al.*, 2012). Studies in dry farmed olive orchards have shown that the higher the tolerance to herbaceous vegetation, the lower the olive yields due to the competition for resources, in spite of some soil fertility parameters could increase with time (Ferreira *et al.*, 2013). The information published to date suggests that cover cropping should continue to be extended in perennial tree crops, to prevent soil erosion and increase soil carbon sequestration, but with necessary care being taken to mitigate the risk of yield reduction.

Annual legumes have gained increasing importance in cover cropping. If the sward is composed or contains legume species, soil available N can be increased. Nodulated legumes are able to access atmospheric N₂ due to the symbiotic relationship that they establish with N-fixing bacteria (Russelle, 2008). Legumes have the potential to provide all the N required for their own growth, as well as transferring N to companion crops in intercropping systems (Hardarson & Atkins, 2003; Pirhofer-Walzl *et al.*, 2012). N fixed by legume species can also be used by a succeeding crop in a crop rotation (Zotarelli *et al.*, 2012; Monday *et al.*, 2013). In perennial tree and shrub crops it is also expected that they can benefit from N fixed by the legume cover crops. Studies from coffee (*Coffea arabica* L.) plantations have shown that the coffee plants take up N previously fixed by an intercropped legume (Snoeck *et al.*, 2000).

Annual pasture legumes have a great potential to be grown as cover crops in olive orchards. However, for the success of a given species or cultivar in a new environment, there is a need to test its adaptability. It should be remembered that the species and cultivars that may be used as legume cover crops were developed for pastoral systems. When they are used as cover crops in orchards, they are usually managed without grazing animal impacts in the form of treading, defoliation and excretion. Thus, the literature available for pastoral systems is of little use when the species/cultivars are used as cover crops. In semi-arid environments, for instance, the precocity of cover crops is expected to be crucial for rainfed managed orchards due to their lower competition for water to the trees. Persistence is also one of the key characters of sown legumes given the importance of self-regeneration of the sward to its economic viability. Previous studies using pasture legumes as cover crops are of great significance (Ovalle *et al.*, 2007; Driouech *et al.*, 2008; Mauromicale *et al.*, 2010) but they are usually of short-term crops and have been focused on several aspects other than precocity or persistence of the sown species.

In the particular case of rainfed olive orchards, the economic aspects are of great relevance. The sector is facing a great challenge of sustainability. Olive yields are low and the olive oil price has stagnated due to the increase in acreage of high-density irrigated orchards. A study including five target areas in Portugal, Spain, Italy and Greece, has shown that the viability of these low intensity production systems is only assured if reduced opportunity costs of family labour are accepted, and olive growing is part-time (Duarte *et al.*, 2008). According to Graaff *et al.* (2008) for the Trás-os-Montes target area of Portugal, three or four tested scenarios point to a high level of abandonment, while in the most positive scenario, the areas under semi-

intensive, low input and organic regimes, have increased. Thus, the possibility of reducing the use of expensive fertilizer-N by growing legume species cannot be ignored.

In this study eleven legume species and cultivars were tested in pure stands in a trial carried out for a relatively long period (four growing seasons) to test their persistence when grown as cover crops in a young rainfed olive orchard managed without grazing animals. Data of groundcover percentage, DM yield and N content in the above-ground biomass were recorded. The precocity of all the sown legumes was also assessed, recording the dates of flowering and seed maturity following a decimal code for growth stages. Thus, the main goal of this work is to get data allowing the determination of what legume species/cultivars are best suited to be grown in rainfed olive orchards.

Material and methods

Study site and experimental plot characterization

On-farm research was carried out in Suções, Mirandela (41.49N, 7.26W, and 300 m alt.), in NE of Portugal. The region benefits from a Mediterranean-type climate, typified by long hot and dry summers and moderately cold and wet winters. The average annual temperature and precipitation (1971-2000) were respectively 14.3°C and 508.6 mm. Meteorological data recorded during the experimental period is presented in Fig. 1.

The field experiments were established in a young (~20 years old) orchard, rainfed managed. The trees are of cv. Cobrançosa and spaced at 7 × 7 m, on a homogeneous slope of ~2%. The soil is a Leptosol originating from a bed-rock of schist. Soil analyses performed before the trial revealed the properties presented in Table 1.

Before the start of the experiments, the farmer used to control the weeds through an annual application of a glyphosate-based herbicide, usually applied in May. The farmer also used to apply the fertilizers beneath the tree canopy at approximate rates of 60 kg N, (P₂O₅) and (K₂O)/ha/yr as a compound NPK (10:10:10) fertilizer. To prepare the plot for the experiment, 1500 kg/ha of lime (88% CaCO₃ and 5% MgCO₃) and 250 kg/ha of superphosphate (18% P₂O₅) were applied. In spite of the natural tolerance to soil acidity of most of the annual pasture legumes (Guo *et al.*, 2012), the application of lime and superphosphate to such acidic soils was expected to favour legume nodulation and N fixation.

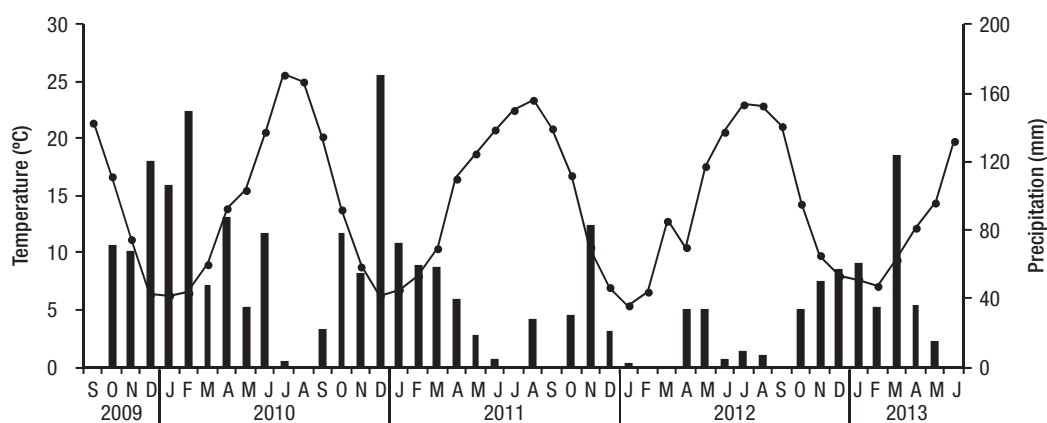


Figure 1. Mean monthly temperature (line) and precipitation (bars) during the experimental period.

Table 1. Selected properties of soils on September 2009 just before the trial start.

Parameters	
Texture (USDA)	Sandy loam
Clay (%)	13.1
Silt (%)	26.6
Sand (%)	60.3
pH (soil:water, 1:2.5)	4.1
Organic C (g/kg) [§]	7.9
Extractable P (mg/kg) [†]	21.0
Extractable K (mg/kg) [†]	96.3
Exchangeable bases [‡]	
Ca (cmol _c /kg)	1.28
Mg (cmol _c /kg)	0.64
K (cmol _c /kg)	0.11
Na (cmol _c /kg)	0.81

[§]Walkley-Black; [†]Egner-Rhiem; [‡]Ammonium acetate pH 7.

Table 2. Legume species, cultivars and seeding rates used in the field trial.

Legume species	Cultivar	Seeding rate (kg/ha)
<i>Ornithopus compressus</i> L.	Charano	15
<i>Ornithopus sativus</i> Brot.	Erica	7.5
	Margurita	7.5
<i>Trifolium subterraneum</i> L.	Dalkeith	25
ssp <i>subterraneum</i> Katzn. and	Seaton Park	25
Morley	Denmark	25
	Nungarin	25
<i>Trifolium resupinatum</i> L. ssp	Prolific	10
<i>resupinatum</i> Gib and Belli		
<i>Trifolium incarnatum</i> L.	Contea	25
<i>Trifolium michelianum</i> Savi	Frontier	10
<i>Biserrula pelecinus</i> L.	Mauro	10

Experimental set up

Pure stands of eleven self-reseeding annual legumes were established in a randomized complete block design with three replications. The area of each individual plot was 49 m² (7 × 7 m), the square defined by four adjacent trees. The total area occupied by the experiment was 1617 m², corresponding to 11 treatments (species and cultivars) and three replications. The species and cultivars used in the study are presented in Table 2. The seeding rates were similar to those prescribed in the region for use in pasture fields. The seeds were broadcast by hand in the corresponding plots and incorporated with a shallow cultivator and a roller.

In a plot next to the main experiment an *in situ* germination trial was carried out to assess seed germination rates and the levels of hardseededness. Thirty three micro-plots of 1-m² each were delimited by wooden slats to receive the 11 species/varieties in

a complete randomized design with three replications. In each micro-plot 300 seeds were sown being covered with a thin layer of soil. The date of sowing was October 14th, 2009. During the autumn and winter after sowing, the seedlings were counted and uprooted as soon as their botanical identification was possible. One year later, also in the autumn and winter, the new seedlings were counted again for determining the amount of hard-seeds germinating one season after sowing.

Field data recovery and laboratory analysis

The ground covered by the sown species relative to weeds and uncovered bare soil was assessed by the pin point method, which measures the proportion of ground occupied by a perpendicular projection on to it of the aerial parts of the plants (Damgaard *et al.*, 2011). A frame with a fixed grid pattern was placed randomly

above the vegetation and a pin was inserted vertically through one of the ten (10 cm spaced) grid points into the vegetation. The first point touched by the pin (sown species, weeds or bare soil) was registered. In each experimental unit the frame was randomly placed in five positions within the available area, registering a total of 50 points per plot in each sampling date. The ground cover percentage by the different components of the sward was determined five to six times a year from late winter to full bloom of the latest flowering species/cultivar. In 2012 and 2013, the dominance of the cover by the sown legumes relative to the weeds was also assessed by separating the legume from the non-legume component from vegetation samples of 0.25 m² cut in spring, close to the maximum growth of the vegetation.

The phenological stage of the legume species was evaluated from late winter to mid-late spring by using the extended BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemical industry) – scale, a system for a uniform coding of phenological growth stages of all mono- and dicotyledonous species (Meier, 2001). The BBCH-scale is a decimal code which is divided into principal and secondary growth stages (Meier, 2001). Taking into account the indeterminate growth habit and abundant branching of most of the species/cultivars used in this study, the BBCH-scale was applied to the plant stand instead of the more usual application to the main stem.

The above-ground dry biomass yield was evaluated in a cut made close to the maximum vegetative growth point of the legume species. The field samples were randomly collected by placing a grid of 0.5 × 0.5 m on the vegetation. In the first two years (2010 and 2011) the samples were oven-dried at 70 °C, ground, weighed and analyzed for elemental composition. In 2012 and 2013 the samples were separated fresh into the legume and non-legume components as already mentioned. The samples were thereafter dried, weighed and analyzed separately. The comparison of the above ground DM yields of the sown and unsown components of the sward complemented the data obtained by the pin-point method.

Tissue analysis was performed by Kjeldahl (N), colorimetry (boron and phosphorus), flame emission spectrometry (potassium) and atomic absorption spectrophotometry (calcium, magnesium, copper, iron, zinc and manganese) methods (Walinga *et al.*, 1989). Nutrient content in the above ground biomass of covers was estimated from DM yield and its nutrient concentration. In 2012 and 2013, both legume and non-legume components were taken into account in proportion to their relative dry biomass and nutrient concentrations, since they were analyzed separately.

Orchard management during the experimental period

In March 2012, a post-emergence herbicide (cy-cloxydim), used for the control of annual and perennial grasses in broad-leaved crops, was applied at a rate of 2 L/ha of commercial product (Focus Ultra, 100 g/L of active ingredient) to reduce the increasing dominance of Rattail fescue [*Vulpia myuros* (L.) C.C. Gmel.] in the covers. Also in March 2012, 2 kg B/ha (as borax, 11% B) and 20 kg of P/ha (as superphosphate 18%) were broadcast in field plots. The application of B and P was also to favour the legume species due to the increasing dominance in the covers by Rattail fescue.

Once a year in the autumn, the farmer sprayed the foliage of the trees with a copper compound to control the olive peacock disease (*Spilocaea oleaginea*). Olive fruit fly (*Bactrocera oleae*) was also controlled every year by spraying the olive trees with a dimethoate-based insecticide. In the spring of 2012, the orchard was subjected to a slight pruning, following the habitual triennial sequence of pruning established by the farmer. Late in spring, after seed maturity, the covers were destroyed with a rotary slasher and left on the ground as a mulch.

Statistical analysis

Comparisons among annual legume species and cultivars regarding DM yield, N concentration and N content were provided by ANOVA. After ANOVA examination, the means with significant differences ($\alpha < 0.05$) were separated by the Tukey HSD test ($\alpha=0.05$). Mean confidence limits ($\alpha=0.05$) were also determined for some graphical representations.

Results

From sowing, on October 14th 2009 to March 30th 2010, the seed germination rates ranged from 5.3% to 48.1% in cvs. Mauro and Margurita, respectively (Fig. 2). Charano also showed a very low germination rate (9.3%). The germination rate of the other species and cultivars ranged from 25% to 40%. In the autumn/winter season of 2010/2011, the emergence from the seeds sown in October 2009 was quite low. Only Charano (8.7%) and Dalkeith (9.3%) had minimally relevant values.

The subterranean clovers Nungarin and Dalkeith started flowering usually in early March and kept blooming until late April (Table 3). Mature seeds began to appear in reasonable amounts from the middle of

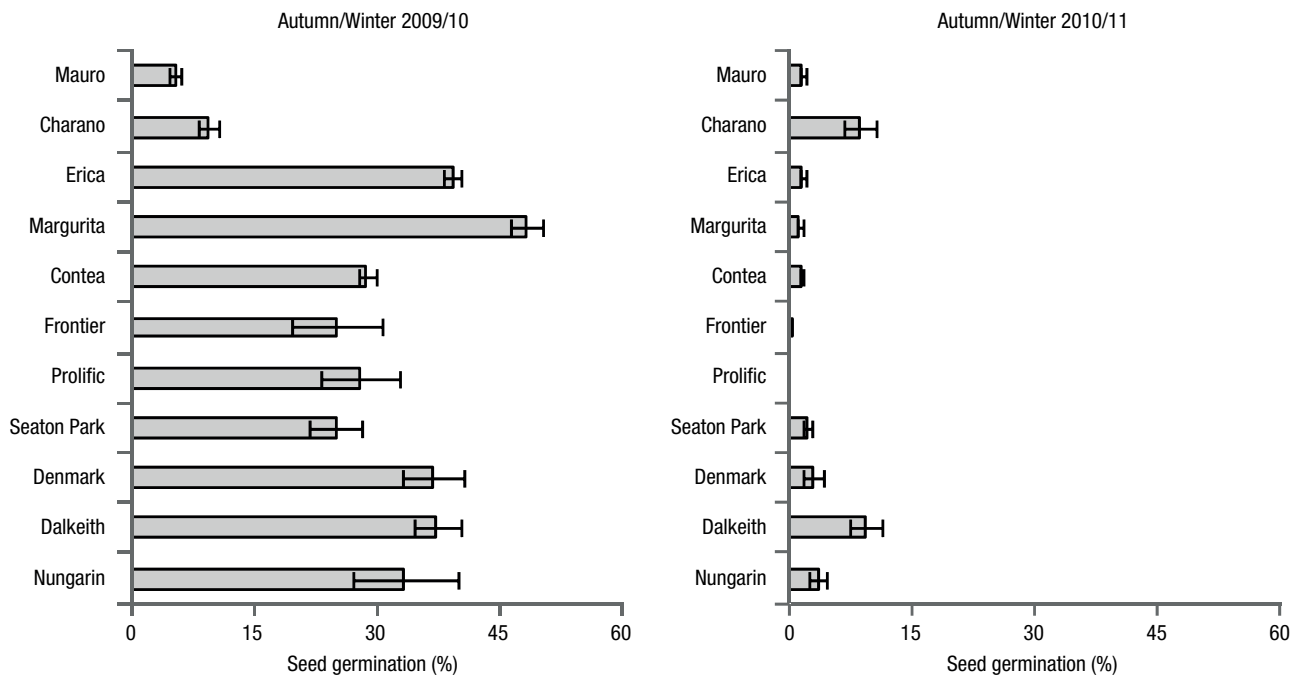


Figure 2. Emergences in the autumn and winter after sowing (left) and one year later (right). Seedlings of the first year were removed after counted. Error bars indicate the mean confidence limit ($\alpha = 0.05$).

Table 3. Average phenological growth stages for the eleven legume species/varieties after four years (2010-2013) of regular observations during the winter/spring period. Phenological growth stages established according to BBCH-scale (Meier, 2001).

	Feb	Mar	Apr	May	Jun
<i>T. subterraneum</i> cv. Nungarin			
<i>T. subterraneum</i> cv. Dalkeith			
<i>T. subterraneum</i> cv. Denmark			
<i>T. subterraneum</i> cv. Seaton Park			
<i>T. resupinatum</i> cv. Prolific			
<i>T. michelianum</i> cv. Frontier			
<i>T. incarnatum</i> cv. Contea			
<i>O. sativus</i> cv. Margurita		
<i>O. sativus</i> cv. Erica		
<i>O. compressus</i> cv. Charano	
<i>B. pelecinus</i> cv. Mauro			

..... Flowering (from beginning to end of flowering applied to plant stand, code 60-69).

- - - - - Seed maturity (first seeds/pods mature to last seeds/pods mature applied to plant stand, code 81-89).

April. Charano started flowering earlier than any of the other species and cultivars. However, Charano showed a pronounced indeterminate flowering habit, producing flowers over a long period. The first mature seeds only occurred from early May. Even so, Charano was the third early-maturing legume in this experiment, following Nungarin and Dalkeith, the earliest maturing ones. The other species and cultivars began flowering usually during April, and significant amounts of mature seeds only appeared with the approach of the end of May.

Most of the sown legumes developed vigorously from the first growing season being dominant over the

natural vegetation. Some species and cultivars reached a groundcover percentage close to 100% by mid-spring (Fig. 3). The great exception was Mauro, which did not recover from the poor emergence (Fig. 2), reaching a maximum ground cover of only 8%. Charano, also having had problems of germination, recovered very well benefiting from its large branching and a very favourable spring. Charano, presented 18% of ground cover on April 1st and 70% one month later on May 1st. In 2011 most species and cultivars reached high groundcover percentages earlier in the spring, probably due to the large seed bank that was formed in the previous year. Mauro showed a slight recovery but maxi-

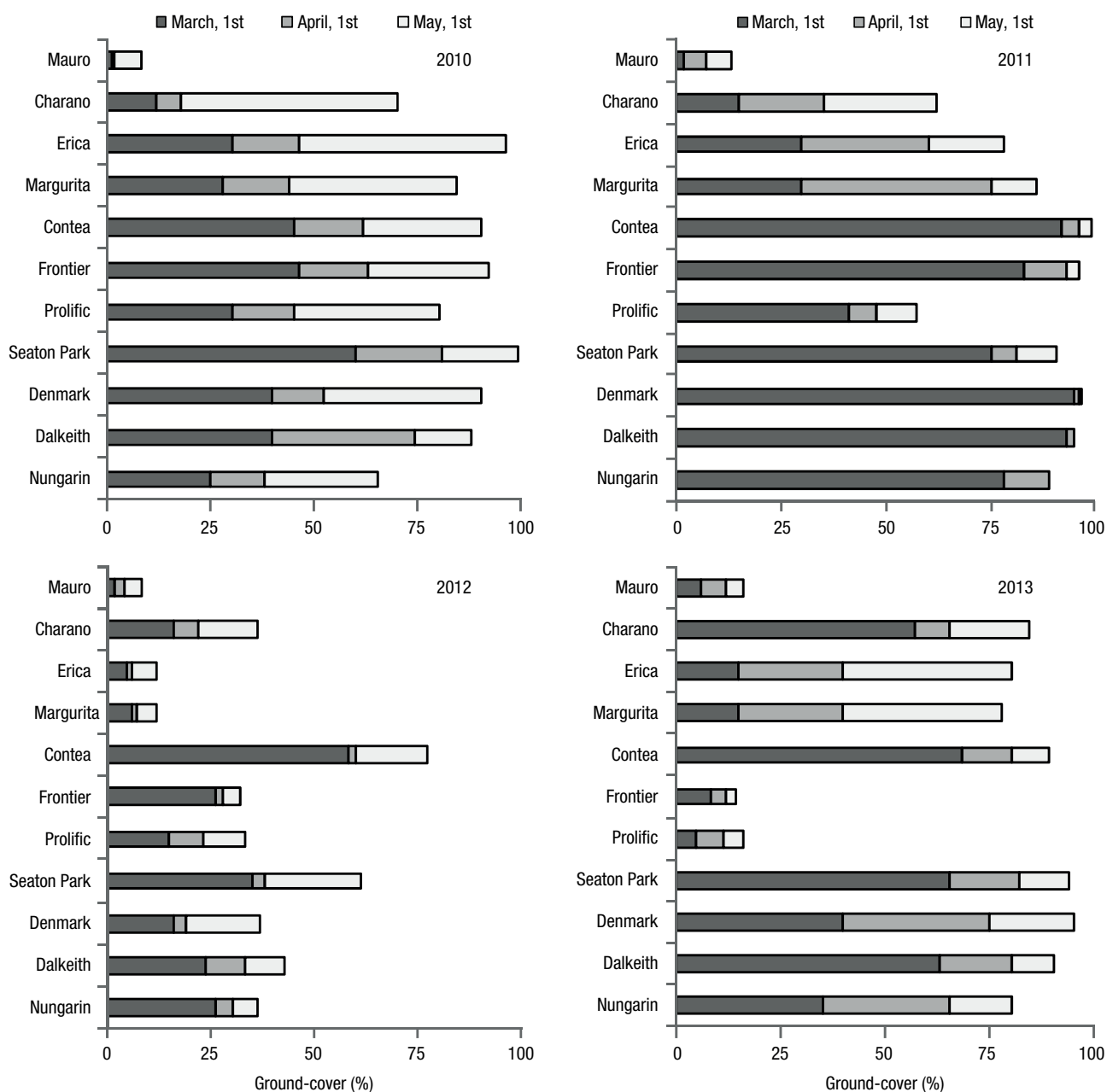


Figure 3. Groundcover percentage by sown legumes during the winter/spring period of the years 2010 to 2013 assessed by the pin-point method.

imum ground cover was only 13%. Prolific plants appeared to be severely damaged by frost in late winter. The groundcover percentage of Prolific was the lowest among the sown legumes except Mauro. The groundcover percentage reduced dramatically for all legume species and cultivars in 2012 in comparison with the two previous years. Erica and Margurita showed particularly low groundcover, both with 12% on May 1st. The values of Mauro continued to be very low (8%). Contea, a cultivar with an erect growth habit and a vigorous vegetative growth, reached the greatest groundcover, with a value of 77% on May 1st. In 2013 high groundcover percentages were recorded again by

most of the sown legumes. Erica and Margurita reached ground covers of 80% and 78% respectively on May 1st. Prolific practically disappeared from the covers, being in the latter years accompanied by Frontier. Their levels of groundcover were only 16 and 14%, respectively. Fatalities from frost persisted over the years. In 2013 Mauro reached its highest level of groundcover (16%) since the beginning of the experiment.

For most of the species and cultivars the higher DM yields were found in the spring of 2010, the first season after sowing (Fig. 4). In this year, mean DM yields varied from 1.3 Mg/ha in Mauro plots to 7.8 Mg/ha in Contea. In the second (2011) and third (2012) growing

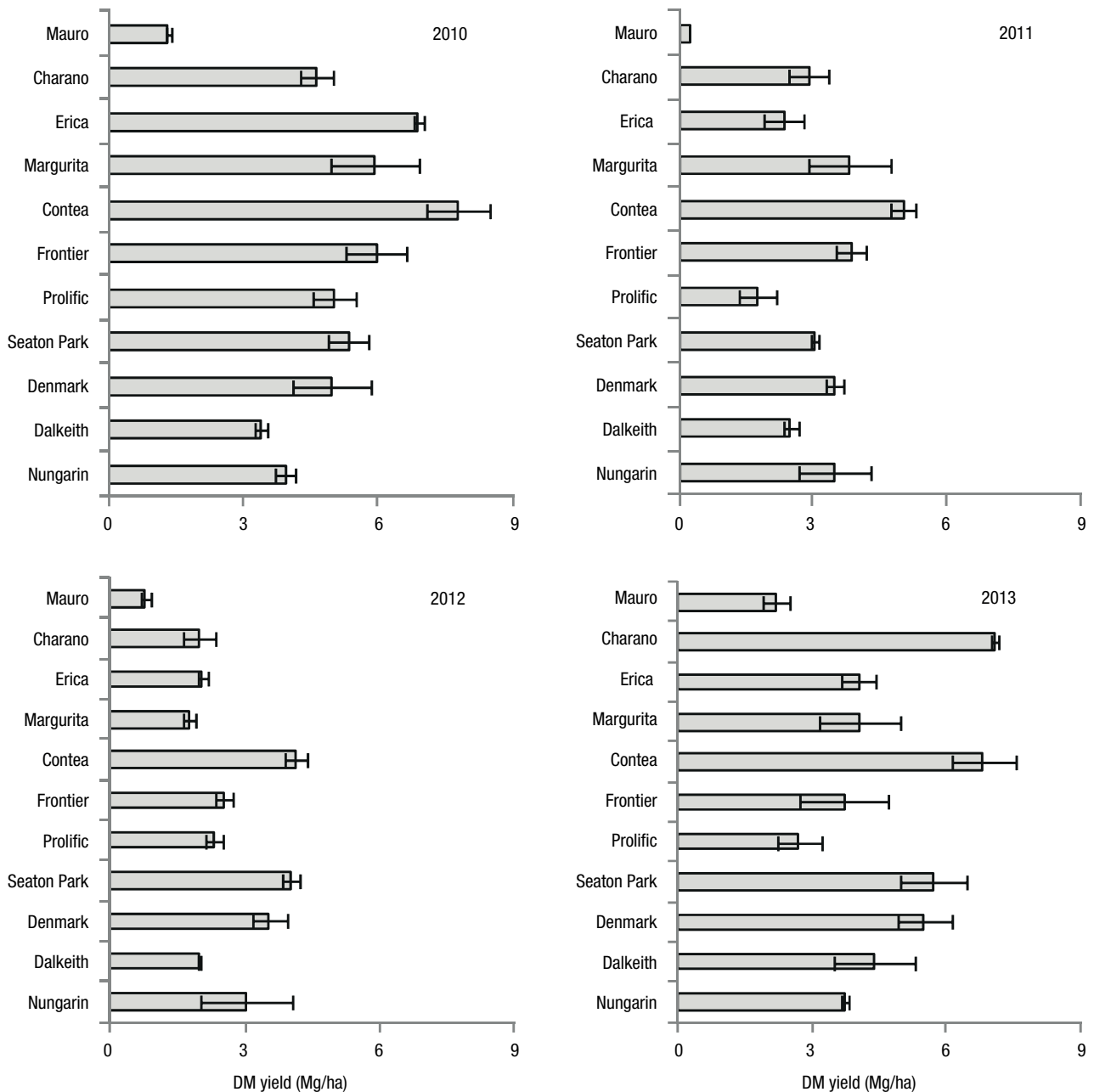


Figure 4. Dry matter yield (legumes + natural vegetation) recorded in the plots of sown legumes in spring of 2010 to 2013. Error bars indicate the mean confidence limit ($\alpha = 0.05$).

seasons DM yields for all legumes species and cultivars were much lower than in 2010. In 2013, DM yields increased again and for some species and cultivars were even higher than in 2010. The low DM yields of Mauro were firstly due to the poor emergence in the first year, but a better performance was recorded in 2013 yielding 0.8 Mg/ha. With reference to Charano, although it showed a poor emergence, the plants seem to have been in a sufficient numbers to ensure high DM yields from the first year. In 2013, Charano was the most productive legume, with an average DM yield of 7.1 Mg/ha. Contea maintained a top position in all the years with DM yields of 7.8, 5.0, 4.0 and 6.8 Mg/ha in the four con-

secutive growing seasons. Prolific had a reasonable performance in 2010 (5.0 Mg/ha) but fell behind as the years progressed. In the last year (2013) the mean DM yield was 2.7 Mg/ha. The early-maturing subterranean clovers, Dalkeith and Nungarin, registered moderately low but relatively stable DM yields. Dalkeith, for instance, produced 3.4, 2.5, 2.0 and 4.4 Mg DM/ha in the four consecutive growing seasons.

N content in the above-ground biomass followed the general pattern observed for DM yield (Fig. 5), the differences partially being justified by the different N concentrations in the plant tissues (data not shown). N content in the above-ground dry biomass were quite

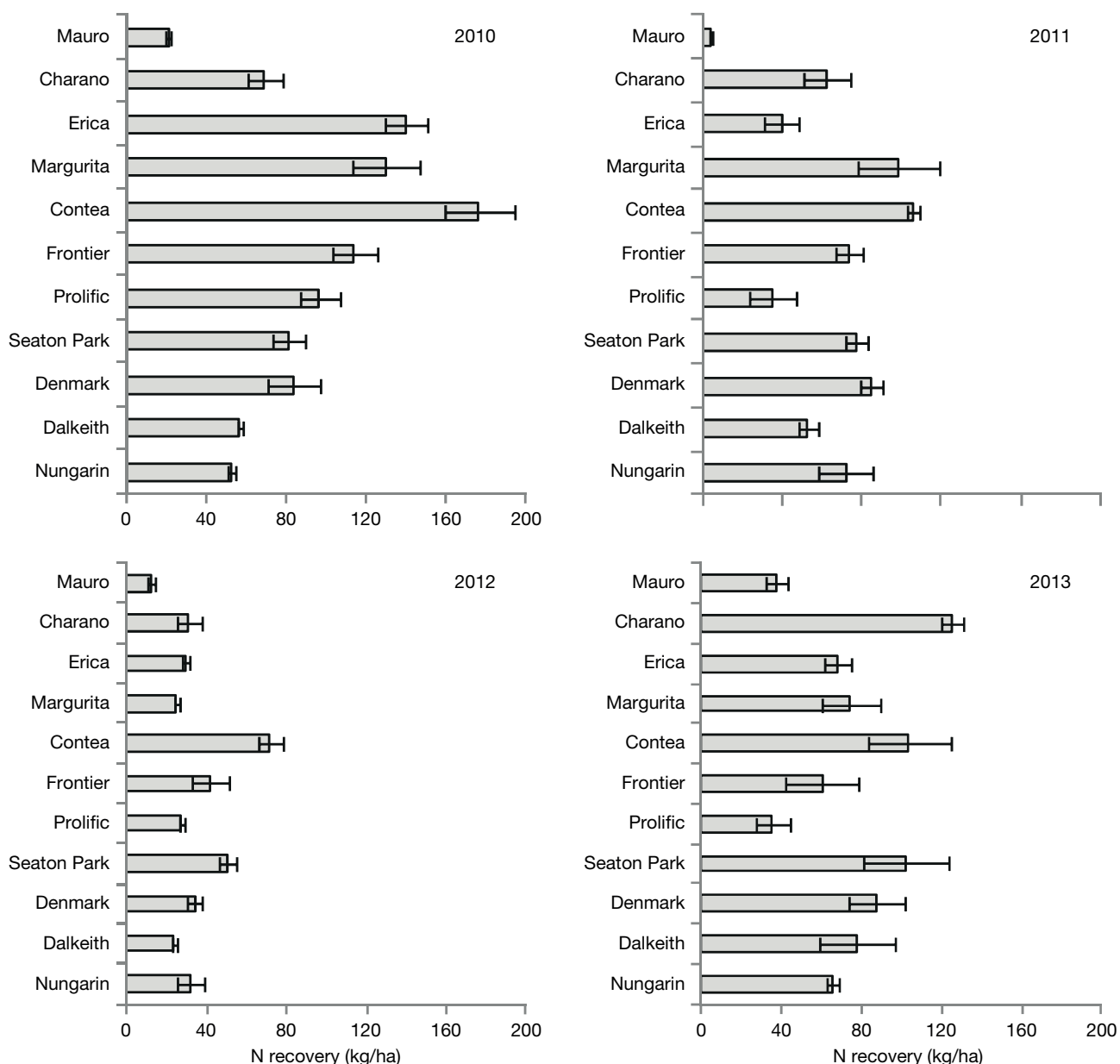


Figure 5. Nitrogen content in above-ground dry biomass (legumes + natural vegetation) in the plots of sown legumes in the spring of 2010 to 2013. Error bars indicate the mean confidence limit ($\alpha = 0.05$).

high in the first growing season (2010), ranging from 20.8 kg N/ha in Mauro to 177.2 kg N/ha in Contea. In the first growing season, Dalkeith and Nungarin, the earliest-maturing and less vegetatively exuberant subterranean clover cultivars, recovered in the above ground biomass, respectively 56.6 and 52.5 kg N/ha. In 2012, N contents in the aerial biomass of all the covers were the lowest of all the four years of the study, with the mean values ranging from 12.2 kg/ha in Mauro and 71.5 kg/ha in Contea. In 2013, N content in the above-ground biomass increased again, reaching a maximum mean value of 124.9 kg/ha in Charano plots. Prolific apparently did not recover from the problems already observed in previous years. Its mean N content

in the above-ground biomass was only 35.0 kg/ha. Dalkeith and Nungarin had fairly high N contents in aerial biomass with average values of 77.2 and 65.0 kg/ha, respectively.

In 2012, the year where the groundcover percentage by the sown legumes was the lowest (Fig. 3), the majority of the biomass produced in each of the plots came from natural vegetation and not from the sown legumes (Fig. 6). The only exceptions were Charano and Contea plots in which the legume component amounted to 65.9 and 58.1%, respectively, of the total dry biomass. The dominant weed in the covers was an annual grass known as Rattail fescue. In 2013, the covers were again dominated by the sown legumes, as had already been

indicated by the data of the groundcover percentage. Expressed in DM percentage of the legume component over the DM of all the vegetation of the plot, the values varied from 65.0% and 90.9%, respectively in Prolific and Denmark plots.

The DM produced in the four growing seasons was statistically different among the different cover crops (Table 4). Contea produced 23.8 Mg/ha whereas Mauro only 4.5 Mg/ha. The subterranean clovers of short growing cycles (Nungarin and Dalkeith) produced DM yields on average lower but not significantly different than those of subterranean clovers of longer growing

cycles (Denmark and Seaton Park). The mean DM yields of serradellas (Charano, Margurita and Erica) were very similar among them, ranging from 15.5 and 16.0 Mg/ha. Prolific produced relatively low biomass due to some problems related to frost observed in the second growing season. N content followed the pattern observed for DM yield. The samples collected in the Contea plots in the four growing seasons amounted to an average N content of 458.1 kg/ha whereas those of Mauro plots only 74.3 kg N/ha. The early-maturing subterranean clovers showed N contents higher than 200 kg/ha, while the N contents of those of longer

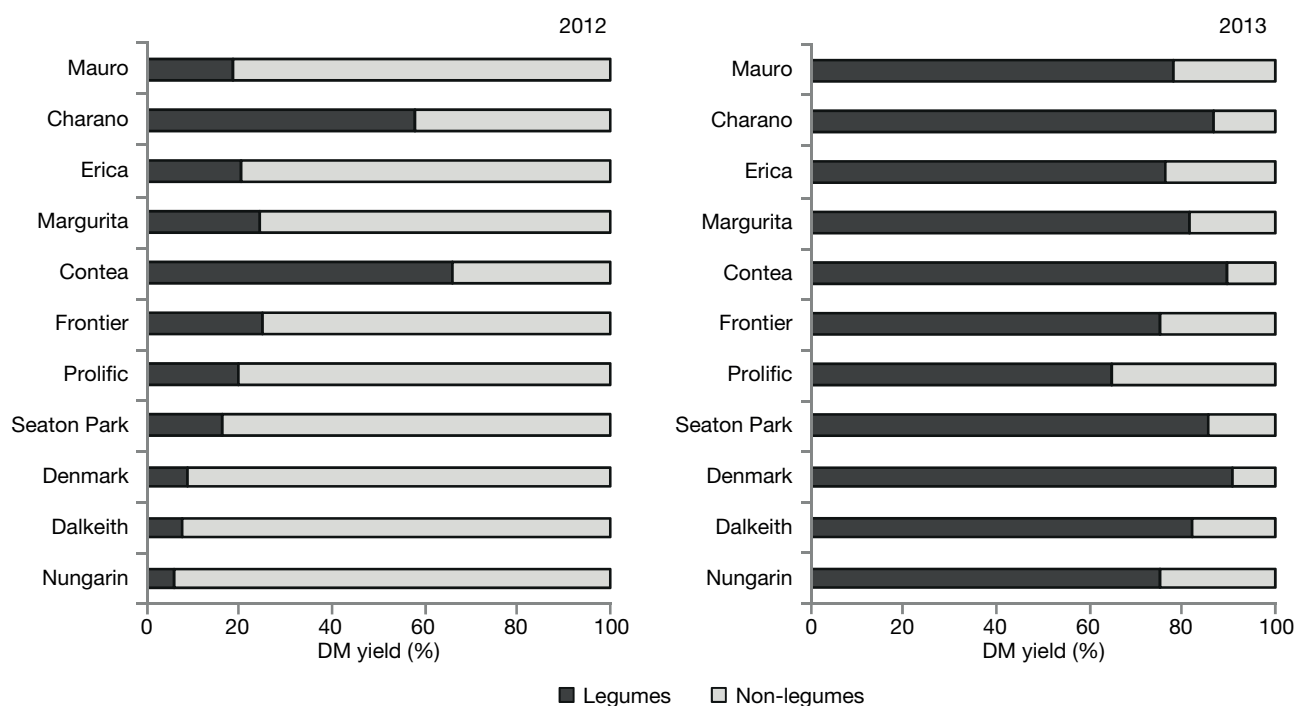


Figure 6. Dry matter yields separated by sown legumes and natural vegetation and expressed as a percentage of the total in the years 2012 and 2013.

Table 4. Accumulated dry matter yield and nutrient content in the above-ground dry biomass in the four growing seasons (2010-2013) of the eleven self-reseeding annual legumes.

	DM yield (Mg/ha)	Nutrient content in above-ground dry biomass					
		N	P	K	Ca	Mg	B
		kg/ha					
Nungarin	14.1 b	220.7 bc	18.7 bcd	184.2 bc	97.9 bcd	27.7 bc	494.8 ab
Dalkeith	12.2 b	210.1 bc	16.0 cd	157.4 c	122.1 abc	31.8 ab	396.1 b
Denmark	17.5 ab	288.9 b	28.3 ab	223.9 bc	142.8 abc	43.2 ab	512.4 ab
Seaton Park	18.1 ab	310.5 b	29.9 ab	255.4 ab	150.3 ab	42.8 ab	557.8 ab
Prolific	11.8 b	194 bc	19.0 bc	183.6 bc	70.6 cd	28.7 bc	365.4 b
Frontier	16.0 b	288.6 b	28.4 ab	256.0 ab	113.8 abc	40.9 ab	531.3 ab
Contea	23.8 a	458.1 a	33.1 a	329.3 a	179.0 a	50.9 a	706.6 a
Margurita	15.5 b	327.3 ab	26.3 abc	216.7 bc	141.4 abc	35.4 ab	479.2 ab
Charano	16.0 b	264.4 b	22.5 abc	190.1 bc	111.5 abc	31.5 ab	397.7 b
Erica	15.9 b	301.2 b	25.3 abc	258.9 ab	128.6 abc	36.8 ab	463.7 b
Mauro	4.5 c	74.3 c	8.5 d	67.4 d	35.5 d	10.2 c	124.7 c

Means followed by the same letter in columns are not statistically different by Tukey HSD test ($\alpha < 0.05$).

growing cycles approached 300 kg/ha. The content of the other nutrients (P, K, Ca, Mg and B) in plant tissues followed the pattern observed for N and DM yield. N being the most limiting factor in this agrosystem, available to nodulated legumes by biological N₂ fixation, the amount of nutrient exported seemed to be proportional to DM yield. The late-maturing species and cultivars, having also higher vegetative exuberance, fixed more N and produced more biomass and, consequently, had a high demand for the other nutrients which were taken up from the soil.

Discussion

All the sown legumes presented germination rates lower than 50%. The result reflects a common feature of the pasture legumes related to their naturally high levels of hardseedness. *B. pelecinus*, cv. Mauro, presented a particularly low germination rate (5.3%) in this study. The high hardseedness of the seeds of bisserula has been frequently reported (Loi *et al.*, 1997; Del Pozo & Ovalle, 2009). Loi *et al.* (1997) found that more than 98% of seeds of several biserrula populations did not germinate after a one-summer exposure on the soil surface. The germination rate of *O. compressus*, cv. Charano, was also very low (9.3%). The high hardseedness of *O. compressus* is also well documented. Ovalle *et al.* (2006) found higher levels of hardseedness in *O. compressus* in comparison with *O. sativus* and *T. subterraneum*.

Charano started flowering earlier than any other legume. However, it presented a very long flowering period. Seed maturity occurred only in May. Margarita and Erica started flowering later and presented also a long flowering period delaying seed maturity to late May and early June. Serradellas had an indeterminate growth habit, great branching and long periods of flowering and pod formation which gave it advantage over the other vegetation if the growing conditions were favourable (Ovalle *et al.*, 2006) as occurred in the springs of 2010, 2011 and 2013. Thus, its precocity was greatly dependent on environmental conditions. Dalkeith and Nungarin were the most precocious (early-flowering and early-seed maturing) cultivars. Mature seeds appeared by the middle of April. The other species and cultivars ranged in flowering and seed maturity from early to mid-season. Most started flowering by April and the first mature seeds appeared by mid-May. The later the occurrence of flowering and seed maturity, the shorter the length of those periods, due to the increase in temperature and rapid decrease in soil available water, due to the onset of summer.

The establishment of the sown legumes and their dominance in the covers were vigorous in the first year.

The regular precipitation and a warm temperature in the autumn favoured seed emergence and the early development of seedlings. The long wet spring that followed increased plant branching and abundant flowering. The low fertility of the soils and the poor competition made by the natural vegetation also favoured the legume component. In this region, farmers restrict the fertilizers to beneath the tree canopy which keeps the fertility of the soil between rows low (Rodrigues *et al.*, 2005). A previous study in a young orchard grown in similar agroecological conditions showed that the natural vegetation reflects the low fertility of the soil. The groundcover percentage between rows was lower than 50% at the maximum vegetative exuberance late in April (Rodrigues *et al.*, 2009). The dominant species were terofites of short growing cycles, such as *Mibora minima*, *Logfia gallica*, *Rumex acetosella* and *Crassula tillaea*, and some perennials such as *Cynodon dactylon*, *Convolvulus arvensis* and *Chondrilla juncea*. Those oligotroph species could not compete with the sown legumes due to their access to atmospheric N₂.

In the second year, the sown legumes showed great development right from the winter, favoured by the large seed bank formed in the previous year. In early March, the groundcover percentage for almost all the sown legumes was already remarkably high, except for Mauro that kept a residual percentage of soil covering. Prolific showed a worse performance in 2011 than in the first year. During the winter, Prolific plants were damaged by winter frost which significantly limited their growth response during the spring.

In the third year there was observed a 'false break', *i.e.*, germination-inducing rainfall events followed by death from severe drought (Nichols *et al.*, 2007). A late-summer rainstorm caused the emergence of sown legumes, which was followed by a very prolonged drought period that led to the death of newly germinated plants. When the rains returned in the autumn, the new wave of germination was reduced further by the fact that weather was already becoming cold. The winter and the early spring were very dry in this year and germinated plants showed no significant development. Legume nodulation, empirically assessed by observing the size and colour of nodules, was clearly unsatisfactory and will have been hampered by the poor environmental conditions. In this year the ground cover was less than 50% for most legume species. Another important aspect in the failure of the legume component will have been the increase in soil available N due to the two excellent years of legume growth. There is abundant literature reporting that while the N levels in the soil are low, this favours legumes, but as N-fixing species increase the soil N levels, this facilitates the development of non-legume species (Munoz & Weav-

er, 1999; Van Riper *et al.*, 2010). In this year, the covers were dominated by Rattail fescue which had benefited from the false break of sown legumes and the increase in soil available N. Rattail fescue is a weedy grass species that is prevalent within no-till/direct-seeded cropping systems where soil disturbance is minimal (Ball *et al.*, 2008; Tarasoff *et al.*, 2013). Rattail fescue populations have expanded dramatically in wheat production systems under no-till practices in USA, Australia and other parts of the world (Tarasoff *et al.*, 2013). It seems that Rattail fescue can also be competitive in non-tilled managed olive orchards depending on the soil available N. Considering the critical situation of the sown legumes in the spring of the third growing season, it was decided to apply an anti-grass post-emergence herbicide to control Rattail fescue.

The fourth year was again very favourable for seed emergence and legume species growth. The seed bank that was created in the first and second years, associated with the hardness of seeds, allowed the recovery of the dominance of the cover by the sown legumes. Mauro, albeit with a low groundcover percentage, showed the highest value ever recorded. Mauro is a species of high DM production and high seed yield (Loi *et al.*, 1997; Del Pozo & Ovalle, 2009), and therefore good persistence (Del Pozo & Ovalle, 2009) which will have allowed a slow recovery of its presence in the covers throughout the years. The presence of Prolific and Frontier in the covers lost importance over the years, apparently due to successive plant damage caused by winter frost.

DM yield and N content in above-ground biomass in the four growing seasons reached the highest average values in Contea, respectively ~ 6 Mg/ha/yr and 115 kg N/ha/yr. The earlier maturing subterranean clover cultivars, Nungarin and Dalkeith, had average DM productions and N contents in plant tissues higher than 3 Mg/ha/yr and 50 kg N/ha/yr. The other *Trifolium* and *Ornithopus* species and cultivars showed intermediate values of DM yields and N contents. Biserrulas are known for high biomass production (Del Pozo & Ovalle, 2009), but in this study the average DM yield and N content were only close to 1 Mg/ha/yr and 18 kg/ha/yr, and even though part of the biomass in Mauro plots had been due to native vegetation. In previous studies on legume management as cover crops in perennial tree crops, authors have paid little attention to DM yield. In pastoral systems, DM yield and N content are quite variable depending on pasture species used and the ecological conditions of each site (Peoples *et al.*, 2001; Ovalle *et al.*, 2006; Boschma *et al.*, 2011; Ates *et al.*, 2013), making it difficult to compare with the values found in the present study. However, the late maturing species and cultivars usually fix more N and

produce more biomass than the early maturing ones (Ovalle *et al.*, 2007) as was also recorded in this study. Peoples *et al.* (2001) reported that N₂ fixation amounts for pasture and crop legumes vary between 20 and 25 kg shoot N for every tone of shoot DM produced. Carlson & Huss-Danell (2003) found 33 to 55 kg N/Mg of DM yield. In 2012 and 2013, where legumes and non-legumes were analyzed separately, the N contained per each tone of dry biomass varied from 40 to 54 kg N in 2012 and 49 to 67 kg N in 2013. This means that N concentration in plant tissues was lower than that found in the referenced studies. The reason might be the low N naturally available in this soil and the high cost in carbohydrates of the N fixed by the legume plants.

The different DM yield and N fixation of the early and late-maturing cultivars may be of particular importance in the soil management of perennial tree crops. In rainfed-managed orchards where yield goal and N demand are low (Rodrigues *et al.*, 2011b, 2012), the earlier maturing cultivars could be the better solution. They introduce enough N into the system and transpire less water during the growing season, in particular late in the spring when soil available water is particularly scarce. In irrigated orchards, the demand for N is higher due to the higher expected yields. Moreover, drought is not a major problem in irrigated conditions. In this context, growing the later-maturing cultivars could be the better decision.

This study provides interesting clues on the management of legume cover crops in olive orchards which may also be of relevance to other fruit trees and vineyards. The most precocious legume species and cultivars seem to be particularly promising for rainfed managed olive orchards. They produce less biomass and fix less N but their growing cycle finishes earlier being almost asynchronous with the active growing phase of the olive trees, which may reduce the competition for water resources. These legumes grow actively from the autumn to mid-spring which coincides nicely with the resting period of olive.

Legume cover crops may also be a sustainable way to increase the acreage of organic farming, which has seen little progress in recent years in the region due to the lack of a natural source of N. Commercial organic additives licensed for organic farming are not a feasible option since they have high prices relative to their fertilizer value (Rodrigues *et al.*, 2006). In addition to N fixed, the covers also provide protection against soil erosion. The seeds germinate with the first autumn rains, maintaining a living mulch during the wet season and a mulching of dead vegetation during the summer.

This work has shown that almost all of the species and cultivars maintained high persistence to the fourth growing season. Good persistence is of particular importance

since it avoids regular seeding and the associated costs. A relevant aspect was the fact that the sown legumes overcame a false break without the need for new seeding. It is also important to notice that some sown legumes, namely Prolific and Frontier, suffered significant damage from winter cold, meaning that they are not suited to climatic conditions similar to those of this region.

References

- Ates S, Lucas RJ, Edwards GR, 2013. Effects of stocking rate and closing date on subterranean clover populations and dry matter production in dryland sheep pastures. *New Zeal J Agr Res* 56(1): 22-36. <http://dx.doi.org/10.1080/00288233.2012.732583>
- Ball DA, Frost SM, Fandrich L, Tarasoff C, Mallory-Smith C, 2008. Biological attributes of rattail fescue (*Vulpia myuros*). *Weed Sci* 56: 26-31. <http://dx.doi.org/10.1614/WS-07-048.1>
- Boschma SP, Crocker GJ, Lodge GM, Harden S, 2011. Evaluation of pasture legumes in northern New South Wales, Australia. *New Zeal J Agr Res* 54(3): 203-213. <http://dx.doi.org/10.1080/00288233.2011.594444>
- Carlsson G, Huss-Danell K, 2003. Nitrogen fixation in perennial forage legumes in the field. *Plant Soil* 253: 353-372. <http://dx.doi.org/10.1023/A:1024847017371>
- Damgaard C, Merlin A, Mesléard F, Bonis A, 2011. The demography of space occupancy: measuring plant colonization and survival probabilities using repeated pin-point measurements. *Methods Ecol Evol* 2: 110-115. <http://dx.doi.org/10.1111/j.2041-210X.2010.00053.x>
- Del Pozo A, Ovalle C, 2009. Productivity and persistence of yellow serradella (*Ornithopus compressus* L.) and biserrula (*Biserrula pelecinus* L.) in the Mediterranean climate region of central Chile. *Chil J Agr Res* 69(3): 340-349. <http://dx.doi.org/10.4067/S0718-58392009000300006>
- Driouech N, Abou Fayad A, Ghanem A, Al-Bitar L, 2008. Agronomic performance of annual self-reseeding legumes and their self-establishment potential in the Apulia region of Italy. 16th IFOAM Organic World Congress, Modena, Italy, June, pp: 16-20.
- Duarte F, Jones N, Fleskens L, 2008. Traditional olive orchards on sloping land: Sustainability or abandonment? *J Environ Manage* 89: 86-98. <http://dx.doi.org/10.1016/j.jenvman.2007.05.024>
- Ferreira IQ, Arrobas M, Claro AM, Rodrigues MA, 2013. Soil management in rainfed olive orchards may result in conflicting effects on olive production and soil fertility. *Span J Agric Res* 11(2): 472-480. <http://dx.doi.org/10.5424/sjar/2013112-3501>
- Gómez JA, Guzmán MG, Giráldez JV, Ferreres E, 2009. The influence of cover crops and tillage on water and sediment yield, and on nutrient, and organic matter losses in an olive orchard on a sandy loam soil. *Soil Till Res* 106: 137-144. <http://dx.doi.org/10.1016/j.still.2009.04.008>
- Graaff J, Zuazo V-HD, Jones N, Fleskens L, 2008. Olive production systems on sloping land: Prospects and scenarios. *J Environ Manage* 89: 129-139. <http://dx.doi.org/10.1016/j.jenvman.2007.04.024>
- Gucci R, Caruso G, Bertolla C, Urbani S, Tatichi A, Esposito S, Servili M, Sifola MI, Pellegrini S, Pagliai M, Vignozzi N, 2012. Changes of soil properties and tree performances induced by soil management in a high-density olive orchard. *Eur J Agron* 41: 18-27. <http://dx.doi.org/10.1016/j.eja.2012.03.002>
- Guo YJ, Li GD, Hayes RC, Dear BS, Price A, 2012. Tolerance of the annual legumes *Biserrula pelecinus*, *Ornithopus sativa*, *Trifolium spumosum*, *T. vesiculosum* and *T. subterraneum* to soil acidity. *New Zeal J Agr Res* 55(1): 1-14. <http://dx.doi.org/10.1080/00288233.2011.626785>
- Hardarson G, Atkins C, 2003. Optimising biological N₂ fixation by legumes in farming systems. *Plant Soil* 252: 41-54. <http://dx.doi.org/10.1023/A:1024103818971>
- Loi A, Cocks PS, Howieson JG, Carr SJ, 1997. Morphological characterization of Mediterranean populations of *Biserrula pelecinus* L. *Plant Breeding* 116: 171-176. <http://dx.doi.org/10.1111/j.1439-0523.1997.tb02173.x>
- Martínez JRF, Zuazo VHD, Raya AM, 2006. Environmental impact from mountainous olive orchard under different soil-management systems (SE Spain). *Sci Total Environ* 358: 46-60. <http://dx.doi.org/10.1016/j.scitotenv.2005.05.036>
- Mauromicale G, Occhipinti A, Mauro RP, 2010. Selection of shade-adapted subterranean clover species for cover cropping in orchards. *Agron Sustain Dev* 30: 473-480. <http://dx.doi.org/10.1051/agro/2009035>
- Meier U, 2001. Growth stages of mono- and dicotyledonous plants. *BBCH Monographs*, 2nd ed. Federal Biological Research Centre for Agriculture and Forestry. BBCH Publ, Germany. 158 pp.
- Monday TA, Foshee WG, Blythe EK, Dozier WA, Wells LW, Sibley JL, Brown JE, 2013. Nitrogen requirements for sweetpotato following a crimson clover cover crop. *Int J Veg Sci* 19: 78-82. <http://dx.doi.org/10.1080/19315260.2012.679354>
- Montanaro G, Celano G, Dichio B, Xiloyannis C, 2010. Effect of soil-protecting agricultural practices on soil organic carbon and productivity in fruit tree orchards. *Land Degrad Dev* 21: 132-138. <http://dx.doi.org/10.1002/ldr.917>
- Moreno B, Garcia-Rodriguez S, Cañizares R, Castro J, Benítez E, 2009. Rainfed olive farming in South-eastern Spain: long-term effect of soil management on biological indicators of soil quality. *Agr Ecosyst Environ* 131: 333-339. <http://dx.doi.org/10.1016/j.agee.2009.02.011>
- Munoz AE, Weaver RW, 1999. Competition between subterranean clover and ryegrass for uptake of ¹⁵N-labeled fertilizer. *Plant Soil* 211: 173-178. <http://dx.doi.org/10.1023/A:1004646319700>
- Nichols PGH, Loi A, Nutt BJ, Evans PM, Craig AD, Penglley BC, Dear BS, Lloyd DL, Revell CK, Nair RM, et al., 2007. New annual and short-lived perennial pasture legumes for Australian agriculture—15 years of revolution. *Field Crop Res* 104: 10-23. <http://dx.doi.org/10.1016/j.fcr.2007.03.016>
- Ovalle C, Arredondo S, Romero O, 2006. Yellow serradella (*Ornithopus compressus*) and pink serradella (*O. sativus*): Two new species of annual legumes for the Mediterranean climate zone of Chile. *Agric Téc* 66 (2): 196-209. <http://dx.doi.org/10.4067/S0365-28072006000200010>

- Ovalle C, Del Pozo A, Lavín A, Hirzel J, 2007. Cover crops in vineyards: performance of annual forage legume mixtures and effects on soil fertility. *Agric Téc (Chile)* 67(4): 384-392.
- Peoples MB, Bowman AM, Gault RR, Herridge DF, Mccallum MH, McCormick KM, Norton RM, Rochester IJ, Scammell GJ, Schwenke GD, 2001. Factors regulating the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. *Plant Soil* 228: 29-41. <http://dx.doi.org/10.1023/A:1004799703040>
- Pirhofer-Walzl K, Rasmussen J, Høgh-Jensen H, Eriksen J, Søegaard K, Rasmussen J, 2012. Nitrogen transfer from forage legumes to nine neighboring plants in a multi-species grassland. *Plant Soil* 350: 71-84. <http://dx.doi.org/10.1007/s11104-011-0882-z>
- Ramos ME, Benítez E, García P, Robles AB, 2010. Cover crops under different managements vs. frequent tillage in almond orchards in semiarid conditions: Effects on soil quality. *Appl Soil Ecol* 44: 6-14. <http://dx.doi.org/10.1016/j.apsoil.2009.08.005>
- Rodrigues MA, Arrobas M, Bonifácio N, 2005. Análise de terras em olivais tradicionais de sequeiro. O efeito da aplicação localizada de fertilizantes. *Rev Ciênc Agrár* XXVIII (2): 167-176.
- Rodrigues MA, Pereira A, Cabanas E, Dias L, Pires J, Arrobas M, 2006. Crops use-efficiency of nitrogen from manures permitted in organic farming. *Eur J Agron* 25: 328-335. <http://dx.doi.org/10.1016/j.eja.2006.07.002>
- Rodrigues MA, Cabanas J, Lopes JI, Pavão F, Aguiar CF, Arrobas M, 2009. Ground cover and dynamic of weeds after the introduction of herbicides as soil management systems in rainfed olive orchards. *Rev Ciênc Agrár* XXXII(2): 30-42.
- Rodrigues MA, Lopes JI, Pavão F, Cabanas JE, Arrobas M, 2011a. Effect of soil management on olive yield and nutritional status of trees in rainfed orchards. *Commun Soil Sci Plant Anal* 42: 993-2011. <http://dx.doi.org/10.1080/0103624.2011.562582>
- Rodrigues MA, Pavão F, Lopes JI, Gomes V, Arrobas M, Moutinho-Pereira J, Ruivo S, Cabanas E, Correia CM, 2011b. Olive yields and tree nutritional status during a four year period without nitrogen and boron fertilization. *Commun Soil Sci Plant Anal* 42: 803-814. <http://dx.doi.org/10.1080/00103624.2011.552656>
- Rodrigues MA, Ferreira IQ, Claro AM, Arrobas M, 2012. Fertiliser recommendations for olive based upon nutrients removed in crop and pruning. *Sci Hort* 142: 205-211. <http://dx.doi.org/10.1016/j.scienta.2012.05.024>
- Sirrine R, Letourneau K, Shennan C, Sirrine D, Fouch R, Jackson L, Mages A, 2008. Impacts of groundcover management systems on yield, leaf nutrients, weeds, and arthropods of tart cherry in Michigan, USA. *Agric Ecosyst Environ* 125: 239-245. <http://dx.doi.org/10.1016/j.agee.2008.01.005>
- Snoeck D, Zapata F, Domenach AM, 2000. Isotopic evidence of the transfer of nitrogen fixed by legumes to coffee trees. *Biotechnol Agron Soc Environ* 4(2): 95-100.
- Tarasoff S, Mallory-Smith C, Ingineri L, 2013. Vernalization responses and subsequent fertility of two climatically distinct populations of raitail fescue (*Vulpia myuros* [L.] C.C. Gmel.). *Weed Biol Manag* 13: 24-30. <http://dx.doi.org/10.1111/wbm.12006>
- Van Riper LC, Larson DL, Larson JL, 2010. Nitrogen-limitation and invasive sweetclover impacts vary between two Great Plains plant communities. *Biol Invasions* 12: 2735-2749. <http://dx.doi.org/10.1007/s10530-009-9678-y>
- Walinga I, van Vark W, Houba V, van der Lee J, 1989. Soil and plant analysis: Part 7 - Plant analysis procedures. Wageningen Agric Univ, The Netherlands. 263 pp.
- Zotarelli L, Zatorre P, Boddey M, Urquiaga S, Jantalia P, Franchin C, Alves R, 2012. Influence of no-tillage and frequency of a green manure legume in crop rotations for balancing N outputs and preserving soil organic C stocks. *Field Crop Res* 132: 185-195. <http://dx.doi.org/10.1016/j.fcr.2011.12.013>