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Field evaluation of selected autochthonous herbaceous species for cover crops in Mediterranean woody crops

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ABSTRACT

This study evaluated under two contrasting field conditions, and during two seasons, four autochthonous species, *Bromus rubens* (Br), *Brachypodium distachyon* (Bd), *Medicago truncatula* (Mt) and *Anthemis arvensis* (Aa), as single species and in mixtures of Br + Mt, Br + Aa and Br + Mt + Aa, to determine their suitability as temporary cover crops (CCs) for Mediterranean woody crops. Br, Bd and Mt reached above 90 % ground cover (GC) in spring, while Aa reached a maximum of 80 %; in early winter GC varied from 0 % to 40 %, approximately, depending on the sowing date in the fall. The thermal time required to reach 30 % GC and seed maturity appeared as two key traits to evaluate the suitability of autochthonous species as temporary CCs for Mediterranean woody crops. Our experiment showed that early seeding (no later than mid-October) is critical to exploit these traits into effective CCs under Mediterranean conditions. Phenologic models developed for the tested species, based on temperature, are capable of predicting their development properly under field conditions, and they might be used to predict the evolution of GC when calibrated from field experiments. Aboveground biomass was affected by edaphic conditions, with higher values being observed in the most fertile soil, in Córdoba, in the range of 300–700 g m^{-2}, compared to Adamuz with 150–350 g m^{-2}. When used as monospecific CCs, Br, Bd and Mt had a higher aboveground biomass than Aa. We observed no differences in aboveground biomass among mixtures within each of the two locations. Differences in fine root biomass (RB) and fine root length density (RL_d) resembled those of aboveground biomass, albeit with a higher variability. When used as monospecific CCs, fine RB and fine RL_d were higher for Br and Bd, followed by Mt, with Aa having the lower values. Fine RB, or fine RL, in the top 0–30 cm of soil is also a relevant trait for selecting temporary CCs for Mediterranean woody crops. Our results show that they are also greatly affected by edaphic-climatic conditions and that they have a moderate correlation with aboveground biomass. The analysis of soil water uptake in the Córdoba plot showed no significant differences among the monospecific species and the mixtures, with ET values during the 2014/15 and 2015/16 growing seasons in the range of 325–300 mm.

1. Introduction

Sustainability of current agricultural systems requires, in many cases, a transition towards management strategies that can provide a balanced approach to the provision of several ecosystem services. Increasing the delivery of critical supporting, regulating and cultural services such as soil conservation and enhancement or carbon sequestration, while maintaining provisioning services such as food production, is one of the current challenges of agriculture worldwide (Millennium Ecosystem Assessment, 2005). This will require the widespread adoption of management practices that can provide multiple benefits when integrated into cropping systems. Using cover crops (CCs) is one of these practices that, despite being known for centuries in agriculture (Pieters, 1927; Worthen, 1948), is gaining relevance to ensure the sustainability of agricultural systems and to provide multiple agroecosystem services (Blanco-Canqui et al., 2015; Daryanto et al., 2018; Islam et al., 2021). There are multiple strategies to implement CCs (e.g., Grant et al., 2006; Clark, 2007; Gómez et al., 2021a) depending, for instance, on the type of main crop (e.g., annual vs. permanent crop; Gabriel and Quemada, 2011; Gómez et al., 2018a), its location in the field (e.g., as a single crop in rotations vs. intercropped with the main crop; Gabriel and Quemada, 2011; Amossé et al., 2014), its duration in

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the field (temporary vs. permanent; Gucci et al., 2012; Guzmán et al., 2019) and its purpose (e.g., green manure or erosion control; Benincasa et al., 2010; Gómez et al., 2018a). These different approaches, combined with the large variety of agricultural soils, climates, and crops in which CCs might be implemented, result in a large number of studies aimed at their evaluation for specific situations (e.g., Bergtold et al., 2019; Florence and McGuire, 2020; Singhal et al., 2020).

Woody crops, such as olive trees, vines, almond trees, orange trees and so on, cover 28 Mha in the EU alone, with the majority (approximately 80 %) located in the Mediterranean area (FAOSTAT, 2022). Under Mediterranean conditions, these crops face serious agro-environmental challenges, such as unsustainable erosion rates and decreasing soil quality and biodiversity (e.g., Scheidel and Krausmann, 2011; Gómez, 2017; Winter et al., 2018; Guerra et al., 2021). Among sustainable management practices in woody orchards, the use of CCs is widely accepted as a management tool that reduces soil losses, improves soil fertility and increases biodiversity (e.g., Biddoccu et al., 2016; Gómez, 2017; Montanaro et al., 2017; Gómez et al., 2018a; Winter et al., 2018). In addition to reducing soil erosion, CCs increase soil organic carbon content and nutrient recycling, thus stimulating biological activity and diversity, and reducing runoff and nitrate leaching (e.g., Steenwerth and Belina, 2008; Moreno et al., 2009; Vicente-Vicente et al., 2016; Novara et al., 2019; Vignozzi et al., 2019). Vegetative cover also allows greater accessibility and ease of transit in orchards in adverse weather conditions (e.g., high soil surface moisture due to rains at harvest time; a common situation in Mediterranean olive groves) and reduces soil compaction caused by machinery traffic (Pardini et al., 2002; Gómez et al., 2009; Gucci et al., 2012; Palese et al., 2014). In semi-arid Mediterranean conditions, due to the risk of competition for soil water with the main crop and of penalty of crop yield (e.g., Gucci et al., 2012; Muscas et al., 2017), the generalized strategy for CCs in woody crops is that of temporary CCs growing during the rainy season (fall and winter) and killed chemically or mechanically in late winter-early spring (Palese et al., 2014; Gómez, 2017; Guzmán et al., 2019). However, in vineyards, some studies have shown that CCs, while reducing wine-grape production due to competition for water and nutrients during the growing season, improved must quality (e.g. by increasing the content of sugar or anthocyanin; Lee and Steenwerth, 2013; Muscas et al., 2017).

Although there are decades of research exploring CC alternatives for Mediterranean tree crops (e.g., Ruiz de Castroviejo, 1969; Scienza et al., 1988), the reality is that currently most of the CCs used are natural vegetation growing spontaneously on farms (i.e., spontaneous CCs). As an example, in Spain spontaneous CCs are used in 1.18 Mha of woody crops, 22.2 % of the area devoted to woody crops, while seeded CCs are used only in 24,066 ha, 0.45 % of the woody crops area (ESYRCE, 2020). This situation, which field studies clearly identify (e.g., Guzmán et al., 2019, in vineyards), occurs simultaneously with the need to improve the species composition and CC management in Mediterranean woody crops to enhance its sustainability and improve the provision of agro-ecosystems services. Several studies have indicated an increase in biodiversity and in natural predators of pests with increased diversity in CCs; e.g., Gómez et al. (2018a) in olive groves or Nunes et al. (2015) in vineyards. Other have pointed out the need to search for new plant species with a short life cycle, better adapted to Mediterranean conditions, so as to reduce the risk of competition for soil water with the main crops and favor self-seeding (e.g., Gómez and Soriano, 2020). Some studies have shown the need to use CC species adapted to high grazing pressure in areas with high rabbit populations (e.g., Carpio et al., 2020). The gradual appearance on the market of new varieties selected for use as CC under Mediterranean conditions (e.g., *Brachypodium*, see Gómez et al., 2019) suggests that there is an interest for enhancing the plant species available to farmers for CCs in Mediterranean woody crops, to fulfill the needs mentioned above. Nevertheless, a better understanding of performance of available species for CCs appears necessary to facilitate their implementation by farmers, thus helping to overcome the significant barriers that remain and impede their widespread adoption. Among them we can mention uncertainty regarding the risk of crop yield reduction, implementation and maintenance costs, the lack of information on the specific management practices or lack of awareness of the potential benefits (Roesch-McNally et al., 2018; Daryanto et al., 2019; Aznar-Sánchez et al., 2020; Gómez et al., 2021b).

In recent years, there have been many studies indicating that incorporating plant functional trait perspective can optimize management improvement in agroecosystems oriented to enhance the provision of ecosystems services (e.g., Wilke and Snapp, 2008; Faucon et al., 2017), some of them oriented to Mediterranean woody crops, such as vineyards (e.g., Garcia et al., 2020a; Guerra et al., 2021). Some studies are aimed at achieving a balance between the species richness of the CC with the provided ecosystem services. Storkey et al. (2015) working on field-crop systems in Wales found an optimum with low-medium levels of species richness (one to four species), and Gómez et al. (2018a) in a 3-year experiment in an olive orchard in southern Spain found similar benefits in reducing runoff and soil losses between seeded homogeneous grass and seeded mix of ten different species. This balance between richness and provision is critical for farmers' adoption, since it can reduce barriers for adoption (e.g., costs, seeding machinery…). Other studies combine field experimentation with modeling, e.g. Garcia et al. (2020b), who determined in an experimental vineyard over two years that the differences in species traits were largely explained by species differences (between 50 % and 70 %) while the remaining differences (between 50 % and 30 %) were explained by seasonal and year variability, as well as intra population variation within species. In other studies, researchers have tried to calibrate models to predict key traits, such as ground cover or biomass (e.g., Bodner et al., 2010; Ramírez-García et al., 2015) or those related to phenological development (e.g., Gómez and Soriano, 2020). The majority of these CC trait-based studies measure them in a single site and soil type over a period of one or two years, or at multiple sites during a single season. While aboveground biomass is key to minimizing soil erosion and reducing nutrient and water runoff losses, belowground biomass is just as or more important for maintaining or improving soil ecosystem services such as soil stabilization, soil-erosion control, nutrient cycling, and soil C accumulation, among others (Wilhelm et al., 2004; Gyssels et al., 2005; Rasse et al., 2005; Blanco-Canqui et al., 2015). It is interesting to note that despite this relevance of root traits for the provision of ecosystem services by cover crops, there is a limited number of experimental studies on root traits, not only under greenhouse conditions, e.g. Hudek et al. (2022), but also under field conditions, e.g. Garcia et al. (2020a).

We present in this manuscript a study aimed at evaluating under controlled field conditions over two consecutive years, and in two different soil types and locations, key traits (phenological development, ground cover, aerial and root biomass, root length density) as well as their impact on soil water content of four annual autochthonous herbaceous species and their low-richness mixes (2 or 3 species) for use as CCs in woody crops under Mediterranean conditions.

2. Materials and methods

2.1. Site, plant species and experiment description

The experiment was carried out during the agricultural years 2014/15 and 2015/16 in the province of Córdoba (southern Spain), in two areas with different edaphoclimatic conditions: in Córdoba (alluvial plain of the Guadalquivir river) at the experimental farm of IFAPA (37.86 N, 4.79 W; 89 m asl), and in Adamuz (Sierra Morena range) on a commercial livestock farm (38.08 N, 4.59 W; 403 m asl) (Fig. 1). The annual means of the maximum and minimum daily temperatures for a twenty-year period (2001–2020) were 24.8 and 25.2 °C, and 11.1 and 10.1 °C in Córdoba and Adamuz, respectively, and the precipitation and annual evaporative demand (ETo as defined by Allen et al., 1998) were 598 and 577 mm, and 1408 and 1212 mm, respectively (IFAPA, 2022).

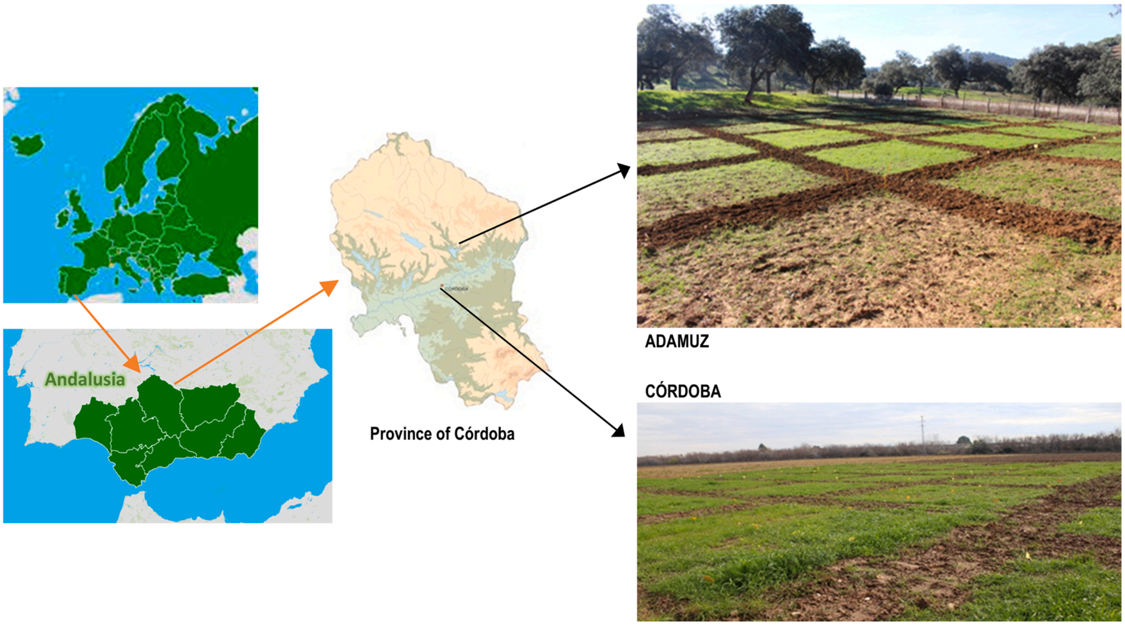

Fig. 1. Location map and view of the two experimental areas.

The soil of the experimental plot in Córdoba was classified as Eutric Fluvisol, according to the FAO system (IUSS, 2015), and had a loamy texture, practically uniform and without apparent restriction for root growth to more than 2-m depth, and a basic pH. The soil of the Adamuz experimental plot had a sandy-loam texture, stony (above 30 % stone content), and with shallow and variable soil depth (from 0.45 to 1.0 m) and a neutral pH, being classified as Eutric Cambisol (IUSS, 2015), see soil properties in Table 1.

Four native annual herbaceous plant species: red brome (*Bromus rubens* L.; Poaceae), purple false brome (*Brachypodium distachyon* (L.) P. Beauv.; Poaceae), barrelclover (*Medicago truncatula* Gaertn.; Fabaceae) and corn chamomile (*Anthemis arvensis* L.; Asteraceae family), and three mixtures of them (*B. rubens* + *M. truncatula*; *B. rubens*+*A. arvensis*, and *B. rubens*+*M. truncatula* + *A. arvensis*) were selected for their field characterization at the two locations and agricultural years. The choice of these plant species was because some of their traits make them very interesting for use as temporary CCs in woody crops under Mediterranean conditions: short life cycle and low plant height as compared to other available species (Hong et al., 2011; Gómez and Soriano, 2020). Additionally, in the case of *A. arvensis*, because it is an unpalatable species, highly resistant to predation by rabbits (Carpio et al., 2017, 2020), thus being considered as a possible CC species in areas characterized by high overgrazing by rabbits, such as many agricultural areas in the Mediterranean (e.g., Barrio et al., 2010; Carpio et al., 2017), and in that of *M. truncatula* for its ability to fix atmospheric nitrogen and its creeping freightage (Gómez and Soriano, 2020).

The seeds of all individual plant species and mixtures of species were sown by hand in autumn (November 9–10, 2014 and November 13, 2015 in Córdoba, and December 1–2, 2014 and October 15, 2015 in Adamuz) in plots (experimental units) of 16 m^2 (4 × 4 m). Single-species and mixtures were grown in two contiguous field sections. Within each section, a randomized block design with three replicates was used. This resulted in 12 plots for single-species and nine for mixtures at each location, maintaining the same distribution in the two growing seasons. The sowing dose was 2.66 g m^{-2} (or approximately 760 seeds m^{-2}) for *B. rubens* (hereafter Br), 3.35 g m^{-2} (or 710 seeds m^{-2}) for *B. distachyon* (Bd), 1.70 g m^{-2} (760 seeds m^{-2}) for *M. truncatula* (Mt), and 0.102 g m^{-2} (860 seeds m^{-2}) for *A. arvensis* (Aa). The dose of each plant species was reduced by half in the Br + Mt (hereafter M1) and Br + Aa (M2) mixtures and by a third in Br + Mt + Aa (M3). A commercial seed company (Cantueso Natural Seeds; https://cantuesoseeds.com/) supplied us with all the seeds, and those of Bd were the mixture of varieties of *B. distachyon* registered with the trade name Vegeta® (Agrosa Semillas Selectas, S.A.). Before sowing, the experimental plots were tilled with a disc harrow followed by a rototiller, incorporating 50 kg ha^{-1} of ammonium sulfate (21 % N) to the soil in the Córdoba plot each season. Fertilizer was not added to the Adamuz plot because it is an organic farm, on which previous livestock activity considerably increased the level of soil organic matter relative to the Córdoba plot (Table 1). Manual weeding was carried out to control weeds, and no irrigation was applied during plant growth cycles.

At the beginning of the trial, just prior to sowing, three composite soil samples (2–4 soil cores each) were taken within each experimental plot at three soil depths (0–20, 20–50 and 50–90 cm) for laboratory analysis. Soil physicochemical analyses were carried out at the Córdoba Agri-Food Laboratory (Regional Government of Andalusia), accredited by ENAC (Spanish National Accreditation Entity). The main soil physicochemical properties in the two experimental plots are given in Table 1. Air temperature (1.2 m above soil surface) and rainfall were automatically recorded at 30-minute intervals using an ECT Air Temperature

Table 1
Mean values of the physicochemical properties of the soil in the two experimental locations (Córdoba and Adamuz).

Soil property	Córdoba			Adamuz		
Depth (cm)	0–20	20–50	50–90	0–20	20–50	50–90
Gravel (> 2 mm) (%)	0.42	0.77	0.79	28.3	36.5	33.2
Clay (%)	14.0	14.5	13.2	10.8	14.0	16.1
Sand (%)	45.9	48.6	52.8	58.9	55.4	56.5
Silt (%)	40.1	36.9	34.1	30.3	30.6	27.4
pH (1:2.5 H_2O)	8.79	8.82	8.80	6.76	6.83	7.39
pH (1:2.5 ClK)	7.96	7.91	7.94	5.55	5.05	5.56
OM^{i} (%)	0.87	0.65	0.55	1.90	0.92	0.67
Organic N^{ii} (%)	0.06	0.05	0.04	0.11	0.06	0.03
Active limestone (%)	3.95	3.77	3.85	0.32	0.29	0.40
CO_3^{2-} (%)	22.9	23.0	23.9	1.18	1.23	1.12
Available K (mg kg^{-1})	248	158	85.0	124.0	84.3	64.0
Exchangeable P^{iii} (mg kg^{-1})	7.97	6.53	4.80	7.93	6.83	6.93
CEC^{iv} (mol$_c$ kg^{-1})	0.110	0.109	0.120	0.210	0.205	0.236

i OM = Organic matter (Walkley-Black method); ^{ii} Kjeldahl method; ^{iii} Olsen method; ^{iv} CEC = Cation exchange capacity.

Sensor (Decagon®) with a radiation shield and a high-resolution rain gauge ECRN-100 (Decagon®), respectively, on each experimental plot during the two experimental years (Fig. 2).

2.2. *Phenology and ground cover data collection*

The phenological stages of the four plant species (Br, Bd, Mt and Aa) were recorded regularly from each plot during the two plant growing seasons (from sowing to senescence) at the two locations, using the BBCH coding system (Hess et al., 1997; Meier, 1997; Meier et al., 2009) to determine the timing of key phenological stages of plant development (i.e., seedling emergence, flowering and seed maturity; Table S1 [Supplementary]). The phenological stages were then related to time, expressed either in number of days (calendar time) or in degree-days (thermal time). Cumulative thermal time (TT; ºCd) was calculated as the sum of the mean daily effective temperatures (i.e., $TT = \sum_0^n \max[0;(T_m - T_b)]$ where T_m is the mean daily air temperature and T_b is the base temperature as °C; Bonhomme, 2000). To calculate the TT, a T_b of 0 °C was used for the four plant species, according to previous calibration for the area (Gómez and Soriano, 2020). A phenological model based on cumulative TT was developed for the four plant species using the Richards' equation (Richards, 1959; Birch, 1999; Gómez and Soriano, 2020).

Evolution of the ground cover for each plant species and mixture of species was recorded every two to four weeks throughout the plant growth cycles by acquiring digital color photographs of the center of each plot. For this purpose, we used a 0.5 × 0.5 m frame provided with a support at 1.35-m to which were fixed a compact digital camera (Canon EOS 100D) and an umbrella to intercept direct sunlight, so that the digital images acquired were orthogonal to the ground and without shadows cast. Percent vegetation cover from digital images was estimated using GreenCropTracker software, which uses a histogram-based threshold method to differentiate gaps from plant tissues (Liu and Pattey, 2010).

2.3. *Aboveground biomass measurement*

Aboveground biomass was measured on two dates during the 2014/15 plant growing season and on a date during 2015/16: April 6–7 (i.e., 148–149 days after sowing; das) and May 16 (188 das), 2015 and April 15–18 (154–157 das), 2016 in Córdoba; and April 25–27 (145–147 das) and May 21 (171 das), 2015 and March 28 (165 das), 2016 in Adamuz. To do this, we cut the vegetation in three subsampling areas of 0.25 m^2 (0.5 × 0.5 m) within each plot on the plant sampling dates of April 2015, and in two subsampling areas in May 2015 and March-April 2016. Plant samples were dried in an oven at 70–75 °C and the dry weight was converted to g m^{-2}. In the CC mixtures (M1–M3), the different plant species were separated from each other prior to introducing them into the oven. For data analysis, the average value of all the subsamples for each plot on each sampling date was used.

The plant samplings carried out in April 2015 (Córdoba and Adamuz) and April 2016 (Córdoba) concurred around the full flowering-beginning of grains development in Br (BBCH 64–71), the middle-final stages of pod development in Mt (BBCH 74–81) and during seed ripening in Aa (BBCH 81–87), with Bd being in a more delayed phenological stage (middle heading-beginning of flowering, BBCH 54–62). In the plant sampling carried out in Adamuz on March 28, 2016, plants of Bd and Br were in the phenological stages of late-booting (BBCH 46–49) and middle-heading (BBCH 54–57), respectively, those of Mt at the beginning of pod development (BBCH 71–73), and those of Aa at the end of seed ripening (BBCH 85–88), (Anon, 1997; Moreau et al., 2006; Hong et al., 2011). On the sampling dates of May 2015, all four plant species were in the final stage of senescence (dead and dry plants; BBCH 97–99) at both locations, although there were still a few greenish-yellow spikes in Bd, both in Córdoba and in Adamuz. The plant samplings carried out in March-April are based on the recommendations for early killing date of temporary CCs to avoid the risk of competition for soil water with woody crops (late March–early April at the latest in Andalusia; Gómez et al., 2014; Alcántara et al., 2017).

2.4. *Fine root sampling and processing*

Dry biomass and length of the fine roots were determined in soil samples taken just after each sampling of the aerial biomass. On each sampling date, soil samples were taken at one sampling point in each plot, at soil depth layers of 0–30, 30–60 and 60–100 cm, using a "Giddings" hydraulic soil corer (3.09-cm-inner diameter) (Giddings Machine Co., Fort Collins, CO) with a rotating head attached to a multipurpose vehicle (John Deere Gator). In the laboratory, each soil-root sample was soaked in water with 5 % sodium hexametaphosphate overnight or 15–20 h to disperse roots and soil particles. The soil-root mix was then placed on a 0.25-mm diameter hole size sieve under running water to repeated washing-out of the soil, and the remaining large plant material and debris were removed from roots sample using tweezers. To improve contrast of living roots in scanned images, cleaned root samples were stained in a Congo red solution (0.9 g l^{-1} water) for 30 min, then

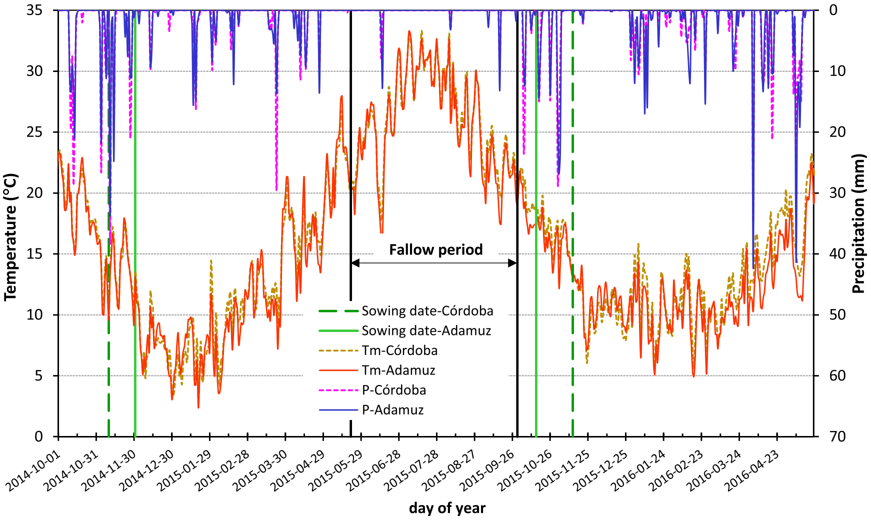

Fig. 2. Mean daily temperature (Tm; °C) and daily precipitation (P; mm) at each location during the two cover crop seasons (from October 2014 to May 2016). Sowing dates: November 9–10, 2014 and November 13, 2015 in Córdoba, and December 1–2, 2014 and October 15, 2015 in Adamuz.

thoroughly rinsed with distilled water and carefully spread on a thin layer of water (2–3 mm) in a transparent waterproof tray (220 × 300 mm). The root samples thus arranged were scanned using a second additional light transparent unit from above (Epson Perfection V800 Photo) at a resolution of 300 dpi, and root images were analyzed for fine ($\leq$ 2 mm in diameter) root length (RL) by using WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Once the roots were scanned, they were carefully collected from the tray (stained and unstained separately), oven-dried at 70 °C and weighed on a precision balance (± 0.1 mg) to determine the fine root biomass (RB) in each soil sample. Fine RL density (RL_d; cm cm^{-3}) and fine RB density (RB_d; mg cm^{-3}) for each soil sample were calculated by dividing root length and root biomass, respectively, by the volume of the soil sample.

2.5. *Cover crops soil water content*

Volumetric soil water content (SWC) was periodically measured down to 1.50 m depth at the center of each plot of the Br and Mt species and of the Br + Mt and Br + Mt + Aa mixtures in the experimental plot in Córdoba, using a neutron probe (CPN, Model 503DR Hydroprobe) previously calibrated in the same soil. The depth intervals of SWC measurements were 0–15, 15–30, 30–60, 60–90, 90–120 and 120–150 cm. Water use by each CC throughout the plant growth cycles was calculated from the soil water balance, as the difference between the SWC readings just before seedling emergence and just after fully seed maturity, plus effective precipitation during their growth cycles. Runoff was assumed negligible, which was considered a reasonable assumption as the slope in the experimental plot was close to zero, no intense rainfall events were registered and no evidence of runoff was observed. Capillary rise was considered negligible (null), due to the absence or too much depth ($>$ 3 m) of the water table. Likewise, the low SWC after the summer, and the experimental years of medium/low rainfall (Fig. 2), suggested that deep percolation would have little relevance, which we confirmed from the analysis of the successive SWC readings in the deeper soil layers.

2.6. *Statistical analysis*

Differences between single-species or between mixtures for each CC characteristic or trait at each measurement date were compared by analysis of variance (ANOVA) as a randomized complete block design. To check the basic assumptions for linear model, two residual diagnostic plots were used. They showed a straight-line fit to the points and a scattering of points with no pattern, fulfilling the ANOVA assumptions of normal distribution of residuals and homogeneity of variances, respectively. The mean values for each CC characteristic were separated by Tukey's HSD test ($\alpha = 0.05$) when ANOVA indicated significant differences between single-species or between mixtures. The same procedures were used to analyze the differences in CC characteristics between measurement dates for each plant species or mixture. Differences in fine root biomass and fine root length density between single-species or between mixtures at different soil depth layers were analyzed as a two-factors (cover crop × soil depth; 4 × 3 or 3 × 3) factorial design at each measurement date. All statistical analyses were performed with Statistix 10 software (Analytical Software, Tallahassee, FL).

3. Results and discussion

3.1. *Phenological development and ground cover by cover crops*

On the experimental plot of Córdoba, with a similar sowing date in the two growing seasons (November 9–10, 2014 and November 13, 2015), seedling emergence (i.e., BBCH 10) was observed 14–15 days after sowing in all plant species in 2014, while in 2015 the period from sowing to emergence was lengthened (21, 24, 26 and 29 days duration in Br, Bd, Mt and Aa, respectively), probably due to lower temperatures in 2015 than in 2014 during the pre- and post-sowing period (mean T in November of 13.9 °C and 12.7 °C in 2014 and 2015, respectively, Fig. 2). Seedling establishment (estimated as initial leaf development, i.e., BBCH 13 in grasses and BBCH 14 in dicots) was reached, in 2014, on December 13 and 16 in Br and Bd, respectively, and on December 21 in Aa and Mt; while in 2015 it was reached on December 21, 23, 25 and 29 in Br, Bd, Aa and Mt, respectively, (Table 2), again slightly later as compared to 2014. Accumulated thermal time (TT, °Cd; with $T_b = 0$ °C) from sowing to seedling establishment was similar in the two growing seasons for each plant species: 390–400 °Cd in *B. rubens*, 435–415 °Cd in *B. distachyon*, 465–435 °Cd in *A. arvensis* and 475–470 °Cd in *M. truncatula* (Fig. 3). In the Adamuz plot, in the 2014/15 season, late sowing (December 1–2) together with low December-January temperatures (mean T of 7.5 ºC; Fig. 2) resulted in a late establishment of seedlings, on January 24 and 31 in Br and Bd, and on February 8 and 12 in Aa and Mt, respectively. In the 2015/16 season, early sowing (October 15) resulted in earlier seedling establishment, on November 10 in Br and Aa, and November 11 and 14 in Bd and Mt, respectively (Table 2).

Flowering and seed maturity dates varied significantly between plant species. Thus, in Córdoba, full flowering (BBCH 65) was reached approximately on March 20 and 11 in *A. arvensis*, on March 31 and 26 in *M. truncatula*, on April 8 and 5 in *B. rubens* and on April 21 and 19 in *B. distachyon* during the 2014/15 and 2015/16 growing seasons, respectively (Table 2). The same chronological order was maintained for the date of fully ripe (BBCH 89): April 17–18 in Aa, April 23–27 in Mt, May 2–3 in Br and May 13–14 in Bd, grouping the two experimental years (Table 2). As for seedling establishment, the TT accumulated from sowing to full flowering was similar in the two growing seasons for each plant species: 1245–1250 °Cd, 1413 °Cd, 1533–1553 °Cd and 1768–1755 °Cd for Aa, Mt, Br and Bd, respectively (Fig. 3). In Adamuz, in the later sowing (2014/15 season) full flowering was delayed until around March 29 in Aa, and April 12, 19 and 30 in Mt, Br and Bd, respectively, while in the early sowing (2015/16 season) full flowering was advanced to February 24 in Aa, March 14 in Mt, and April 6 and 16 in Br and Bd, respectively, and fully ripe was reached on March 30, April 11 and 26, and May 10 for Aa, Mt, Br and Bd, respectively (Table 2).

For the sowing dates carried out in Córdoba (November 9–13), in the 2015/16 season all single-species and mixtures reached ground cover (GC) percentages greater than 80 % in mid-February, with averages of 50 % GC in the second half of January (January 12–27). In the 2014/15 season, the GC was slightly lower (20 % in Aa, 31–35 % in Mt-Bd, and 50 % in Br and mixtures, at the end of January) (Fig. 4a, c), probably related to lower temperatures during December and January in 2014/15 (mean T of 7.8 and 6.9 °C, respectively) than in 2015/16 (10.2 and 10.6 °C), since the rainfall was favorable for CC establishment and growth during the two growing seasons (Fig. 2). In Adamuz, late sowing in 2014/15 (early December) caused a delay in emergence and very low ground cover during winter (5 % GC in Mt and Aa, and around 10 % GC in Bd and mixtures, in early February), although *B. rubens* showed significantly faster and greater GC than the rest of the CCs (20 % GC in early February and 90 % GC in early April), (Fig. 4b). On the contrary, bringing the sowing date forward from early December into mid-October in 2015/16 resulted into a faster GC in autumn, reaching GC greater than 50 % in the multi-specific plots and around 70 % in the mono-specific in mid-December (without significant differences between single-species or mixtures), and all single-species and mixtures reaching 80 % GC in mid-January (Fig. 4b, d).

The analysis of phenological development indicated that the year and date of sowing, and the location or soil type, did not modify the relative order of the four plant species in relation to the dates of flowering and seed maturity, thus, the shortest life cycle was that of *A. arvensis*, followed by *M. truncatula* and *B. rubens*, with *B. distachyon* being the latest. This same order in the duration of the growth cycles was observed for Aa, Mt and Br in a two-year trial in pots under irrigation, in two different locations in Córdoba province (Gómez and Soriano, 2020).

Table 2
Average time to key phenological stages of plant development (i.e., seedling emergence and initial leaf development, flowering and seed maturity) for the four plant species studied (Br, Bd, Mt and Aa) in the two locations and during the two cover crop seasons, expressed in calendar time, as day of the year, DOY, and as days after sowing (DAS, within parenthesis).

Location	Sowing date	Plant species	Seedling emergence Establishment BBCH codes = 10; 13,14[i]	Full flowering BBCH codes = 63–67	Seed maturity (fully ripe) BBCH code = 89
			DOY (DAS)	DOY (DAS)	DOY (DAS)
Córdoba	Nov 9–10, 2014	*B. rubens* (Br)	Nov 24, 2014 (14)[ii] Dec 13, 2014 (33)	Apr 6–11, 2015 (147–152)	May 3, 2015 (174)
		B. distachyon (Bd)	Nov 24, 2014 (15) Dec 16, 2014 (37)	Apr 19–25, 2015 (161–167)	May 13, 2015 (185)
		M. truncatula (Mt)	Nov 24, 2014 (15) Dec 21, 2014 (42)	Mar 30-Apr 2, 2015 (141–144)	Apr 23–26, 2015 (165–168)
		A. arvensis (Aa)	Nov 24, 2014 (14) Dec 21, 2014 (41)	Mar 16–24, 2015 (126–134)	Apr 18, 2015 (159)
	Nov 13, 2015	*B. rubens*	Dec 4, 2015 (21) Dec 21, 2015 (38)	Apr 3–8, 2016 (142–147)	May 2, 2016 (171)
		B. distachyon	Dec 4–7, 2015 (21–24) Dec 23, 2015 (40)	Apr 17–21, 2016 (156–160)	May 14, 2016 (183)
		M. truncatula	Dec 9–11, 2015 (26–28) Dec 29, 2015 (46)	Mar 23–29, 2016 (131–137)	Apr 24–27, 2016 (163–166)
		A. arvensis	Dec 12–14, 2015 (29–31) Dec 25, 2015 (42)	Mar 7–16, 2016 (115–124)	Apr 17, 2016 (156)
Adamuz	Dec 1–2, 2014	*B. rubens* (Br)	Dec 22, 2014 (21) Jan 24, 2015 (54)	Apr 16–22, 2015 (136–142)	May 13–14, 2015 (163–164)
		B. distachyon (Bd)	Jan 6, 2015 (36) Jan 31, 2015 (61)	Apr 28-May 1, 2015 (148–151)	May 18–20, 2015 (168–170)
		M. truncatula (Mt)	Jan 17, 2015 (47) Feb 11, 2015 (72)	Apr 10–14, 2015 (130–134)	May 8, 2015 (158)
		A. arvensis (Aa)	Jan 17, 2015 (47) Feb 8, 2015 (69)	Mar 28–31, 2015 (117–120)	May 1, 2015 (151)
	Oct 15, 2015	*B. rubens*	Nov 2, 2015 (18) Nov 10, 2015 (26)	Apr 4–9, 2016 (172–177)	Apr 26–27, 2016 (194–195)
		B. distachyon	Nov 2, 2015 (18) Nov 11, 2015 (27)	Apr 14–19, 2016 (182–187)	May 9–11, 2016 (207–209)
		M. truncatula	Nov 4, 2015 (20) Nov 11–14, 2015 (27–30)	Mar 10–18, 2016 (147–155)	Apr 10–12, 2016 (178–180)
		A. arvensis	Nov 1–2, 2015 (17–18) Nov 10, 2015 (26)	Feb 21–28, 2016 (129–136)	March 30, 2016 (167)

[i] BBCH codes: 10 = first leaf emerged aboveground (Br and Bd) or cotyledons completely unfolded (Mt and Aa); 13 = 3 leaves unfolded (Br and Bd), and 14 = 4 true leaves unfolded (first unifoliate and three subsequent trifoliate in Mt, and second pair in Aa).
[ii] The first date corresponds to the BBCH 10 (seedling emergence) and the second to the BBCH 13 or 14 (initial leaf development or seedling establishment).

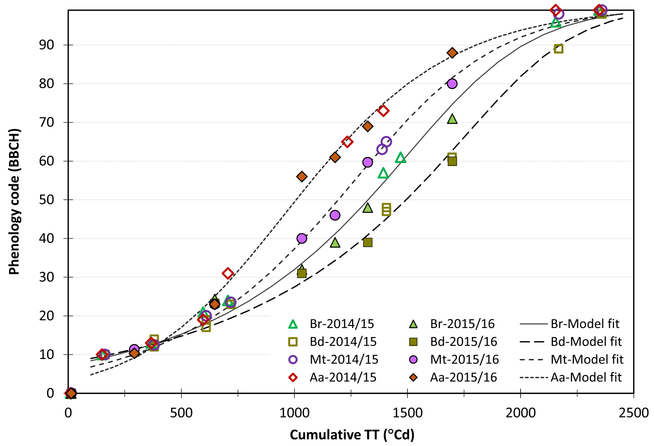

Fig. 3. Cumulative thermal time (TT, °Cd; $T_b = 0$ °C) for the phenological development stages (BBCH codes) of *B. rubens* (Br), *B. distachyon* (Bd), *M. truncatula* (Mt) and *A. arvensis* (Aa) observed in Córdoba during the 2014/15 and 2015/16 seasons. Symbols show the observed values and lines show the fitted models, according to the Richards' equation. Comparison of the observed vs. predicted phenology codes resulted in Pseudo-R^2 values of 0.989–0.995 (n = 16), and RMSE of 3.6–2.6 BBCH values. Sowing dates: November 9–10, 2014 and November 13, 2015.

The inter-annual or inter-location variations in the flowering and seed maturity dates of each CC, as well as in the GC, were due to changes in sowing dates, with an advance of the phenology and a faster and greater GC in winter with the earliest sowing (October 15), and poor establishment and poor GC during winter with the latest sowing (December 1–2) (Table 2 and Fig. 4). As a result, there was a significant effect of advancing the seeding date in the fall, reaching earlier the key phenological stages for properly acting as temporary, self-seeding CCs in Mediterranean conditions. This is particularly relevant to achieve an early development in late fall-early winter, the most erosive period, and seed maturity before the onset of the dry season, which occurs in mid-late spring. These results emphasize the importance of sowing CCs as early as possible in the fall, which in Mediterranean-climate regions will depend yearly on the first significant rainfall (e.g., established in at least 25 mm on four consecutive days after September 1, which on average occurs around mid-October throughout Andalusia; Gómez and Soriano, 2020). Early sowing not only results in earlier seedling emergence and greater soil protection against the risk of water erosion during the rainy season, but also the plants will accumulate the thermal requirements to complete their growth cycles earlier in the spring, which will reduce competition of CCs for soil water with woody crops. This was reached for the single species, Aa, Mt and Br, in our experiment in the year with seeding in mid-October, in line with the predicted average maturity dates by phenologic models for these species (Gómez and Soriano, 2020). Therefore, the date when the plant species reach physiological maturity is a key trait for the optimal management of

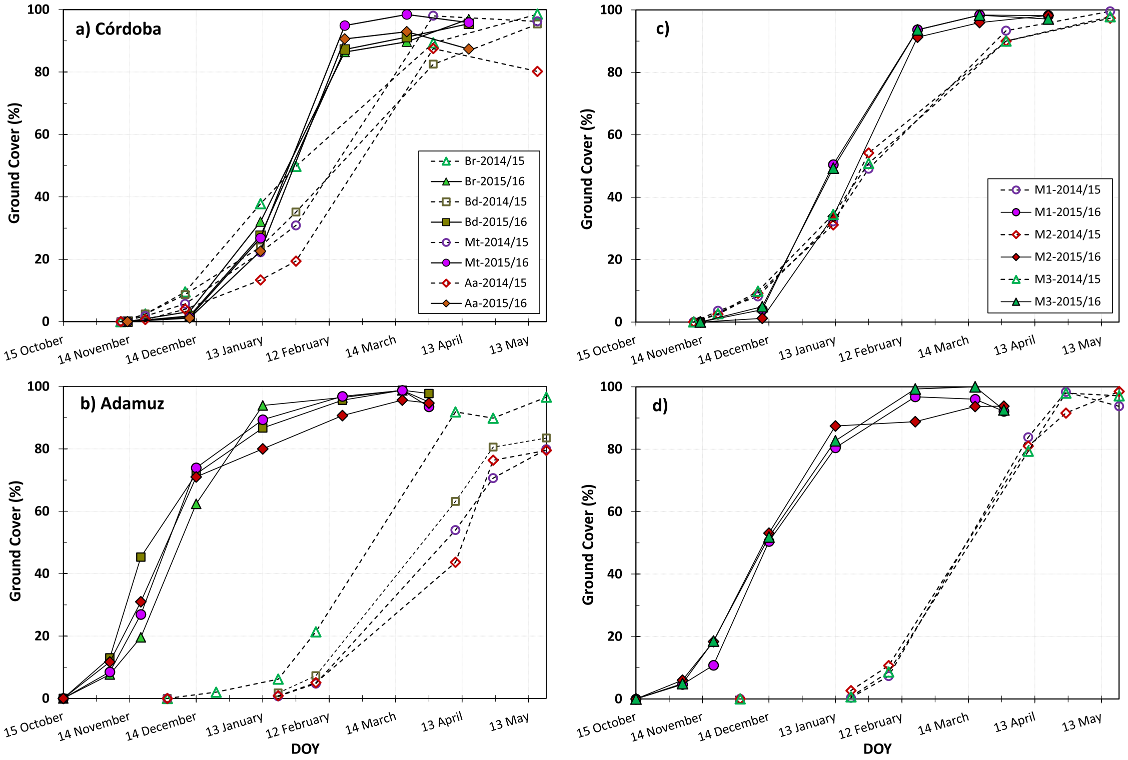

Fig. 4. Evolution of the ground cover of mono-specific, a) and b) left side, and multi-specific, c) and d) right side, cover crops, in the experimental plots of Córdoba, a) and c) above graphs, and Adamuz, b) and d) below graphs, throughout the 2014/15 and 2015/16 growing seasons. The standard errors were, on average, 3.4 % and 2.6 % in Córdoba, and 3.6 % and 6.1 % in Adamuz, in the 2014/15 and 2015/16 seasons, respectively.

temporary, self-seeding, CCs in Mediterranean areas, which requires a fine adjustment of killing date of each CC species to the climatic conditions of each location every year, to avoid their potential adverse effects on soil-water availability and woody-crop yield, and simultaneously allow seed production for self-seeding the following autumn (Unger and Vigil, 1998; Alcántara et al., 2011; Gómez et al., 2014; Gómez and Soriano, 2020). As already mentioned about, prediction of seed maturity date, and other key phenological stages (e.g., seedling emergence, flowering), of CC species at different locations and years can be determinates using validated thermal-time-based phenology models (Fig. 3), such as those developed for *B. rubens*, *M. truncatula* and *A. arvensis* by Gómez and Soriano (2020), which seemed to work properly under our conditions, although there are other alternatives like, for instance, photo-thermal phenology models (e.g., Qi et al., 2000; for legume cover crops). Our results also show that the evolution of the GC percentage from seedling emergence to maximum coverage could be explained by sigmoid growth functions (Tsoularis and Wallace, 2002; Bodner et al., 2010) degree-day-based, following the procedure developed in Gómez and Soriano (2020), or as in Ramírez-García et al. (2015). The thermal time required to reach 30 % GC (Quinton et al., 1997) could be a good indicator of a trait for significant early coverage plant species, which could be estimated according to local climate conditions, based on easily available weather data. For the experimental conditions of this study, this 30 % GC percentage was reached before the onset of winter by the four plant species and the three mixtures evaluated, when sowing was carried out early in the fall (October 15). However, for grass species, *B. rubens* had an earlier emergence and earlier seed maturity date than *B. distachyon*, showing Br advantage over Bd for its implementation as CC in woody crops in Mediterranean conditions in this trait. For all the CCs evaluated, mixtures of Br with Mt or with Mt and Aa (with shorter growth cycles) could be better CC options, since these low-species richness mixes should potentially improve the provision of ecosystem services in these woody crop systems, through increasing biodiversity and landscape values (Storkey et al., 2015; Gómez et al., 2018a), symbiotic nitrogen fixation (case of Mt) and by attracting bees and other pollinator insects (Aa).

3.2. *Aboveground biomass*

In Córdoba, aboveground dry biomass in early spring (April 6, 2015 and April 15, 2016) was similar ($p > 0.05$) in *B. rubens* (582 ± 55 and 620 ± 69 g m^{-2}, respectively; mean ± SD), *B. distachyon* (544 ± 117 and 470 ± 113 g m^{-2}) and *M. truncatula* (668 ± 182 and 540 ± 140 g m^{-2}), and significantly lower ($p < 0.01$) in *A. arvensis* (365 ± 38 and 263 ± 38 g m^{-2}) than in Mt and Br including the two April samplings. There were no significant differences in aboveground biomass between the two samplings in any of the four single-species CCs (Fig. 5a). Comparing among species, in the final sampling of the 2014/15 season (May 16, 2015), the biomass harvested was significantly higher in *B. distachyon* (659 ± 215 g m^{-2}) than in the other three plant species (379 ± 64, 273 ± 114 and 293 ± 128 g m^{-2} for Br, Mt and Aa, respectively), possibly due to the longer life cycle of Bd and the coincidence of sampling with the onset of senescence of Bd plants, while the other plant species had already completed their life-cycle (fully ripe stage; BBCH 89) between two (Br) and four (Aa) weeks before plants sampling (Table 2), and also a large part of their leaves (e.g., Mt) would have already fallen to the ground and begun to decompose. Regarding the comparison among CC mixtures, the aboveground biomass of the three mixtures (M1–M3) was similar ($p > 0.05$) in each of the three sampling dates carried out (Fig. 4c), and did not differ with respect to the biomass of single-crops of *B. rubens* and *M. truncatula* in any of the samplings, but all mixtures produced significantly more biomass that single-crop of *A. arvensis*. Averaging the two samplings carried out in April (after reaching full flowering in the three species of the mixtures), the mean total aboveground biomass was 621, 622 and 687 g m^{-2} for M1 (Br + Mt), M2 (Br + Aa) and M3 (Br + Mt + Aa), respectively. However, the biomass of the different plant species in the mixtures varied significantly, with *A. arvensis* reaching very low percentages

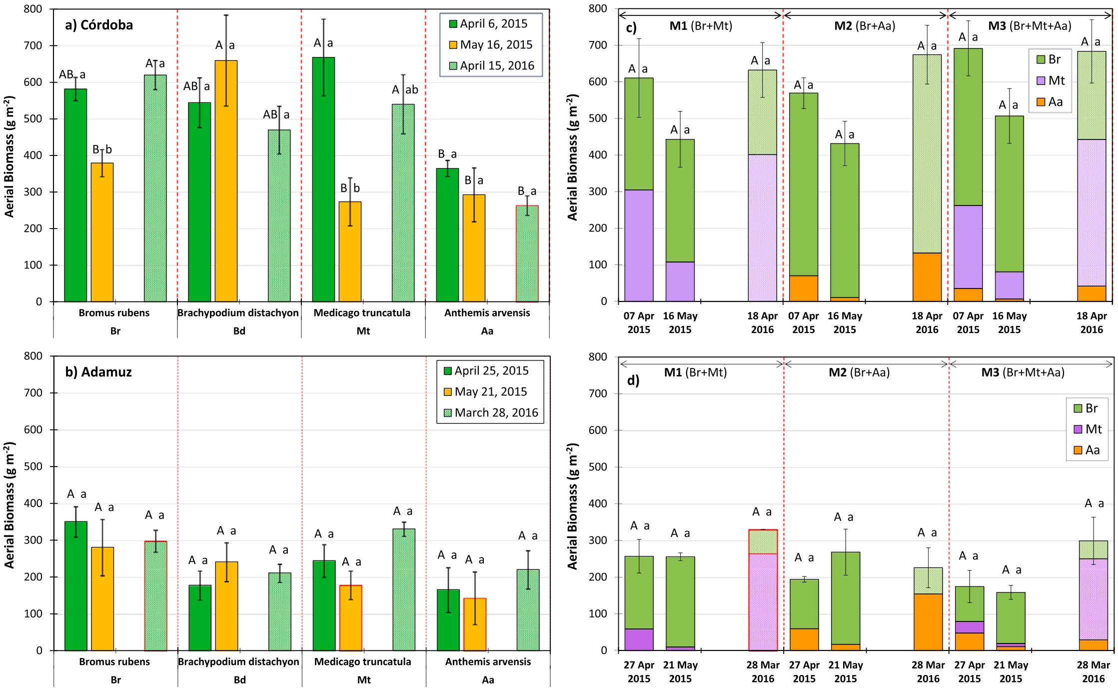

Fig. 5. Mean aboveground biomass (dry matter; g m^{-2}) of the mono-specific, a) and b) (left), and multi-specific, c) and d) (right), cover crops, in the experimental plots of Córdoba, a) and c) (above), and Adamuz, b) and d) (below), for the three dates on which the plant samples were collected at each location (growing seasons 2014/15 and 2015/16). Vertical bars represent standard errors (for total biomass in multi-specific cover crops). Different capital letters indicate significant differences between plant species or mixtures for each sampling date, while different lowercase letters indicate significant differences between sampling dates for each plant species or mixture, according to Tukey's HSD test ($p < 0.05$).

(means of 15.9 % and 5.6 % of the total biomass in M2 and M3, respectively, in April, and 2.4 % and 1.2 % in May, already after four weeks of fully ripe in Aa), while *B. rubens* and *M. truncatula* reached similar aboveground biomass percentages in M1 (means of 43.3 % and 56.7 %, respectively) and M3 (48.7 % and 45.7 %) in April, but with a significant decrease in Mt biomass in mid-May (24.3 % and 14.7 % in M1 and M3, respectively) (Fig. 5c), which was associated with the shorter cycle of Mt and the rapid fall and decomposition of dead leaves in this legume species (Hobbie, 1992; Paul, 2007).

However, in Adamuz, aboveground biomass was similar ($p > 0.05$) for the four single-species and the three mixtures in each of the three plant sampling dates, with no significant differences between sampling dates for each of the CCs (Fig. 5b, d). Nevertheless, when all three plant-sampling dates were included, mean aboveground biomass was significantly lower in Aa than in Br, and in M3 and M2 than in M1. In mono-specific CCs, the aboveground biomass grouping the three samplings varied between 281–351 g m^{-2} in Br, 178–241 g m^{-2} in Bd, 179–331 g m^{-2} in Mt and 143–220 g m^{-2} in Aa, with the highest values at the end of April 2015 in Br and at the end of March 2016 in Mt and Aa, while Bd reached the highest aboveground biomass at the end of May 2015 (Fig. 5b), when the sampling coincided with the end of its life cycle, similar to what was already observed in the Córdoba plots. In the CC mixtures, the mean total aboveground biomass in the three sampling dates was between 255–330 g m^{-2} in M1, 194–268 g m^{-2} in M2 and 158–299 g m^{-2} in M3, with the fraction of the total biomass of each species in the mixtures varying according to the plants sampling date. Thus, in the samplings carried out in the 2014/15 season, Br monopolized the composition of the mixtures, with percentages of the total aboveground biomass of 77 %, 69 % and 55 % in M1, M2 and M3, respectively, at the end of April, and increasing to almost 100 % (96 %, 94 % and 88 % Br in M1, M2 and M3) in the May sampling (Fig. 5d), carried out approximately three, two and one weeks after fully ripe was reached in Aa, Mt and Br, respectively. However, in the sampling carried out at the end of March 2016, Br only represented 20 %, 32 % and 16 % of the total biomass in M1, M2 and M3, respectively, which was attributed to the fact that on that date Aa and Mt were already in an advanced phase of their growth cycles (end of seed ripening and pod development, respectively) while Br was still in an early phase of its growth cycle (panicle emergence). Therefore, mixtures of low plant species number but with species from different families and different life cycle lengths, as in these CC mixtures, would display quantitative and qualitative differences at different dates, adding seasonal visual variation to agricultural landscape values (Storkey et al., 2015).

Aboveground biomass production by the different CCs (mono- and multi-specific) was generally higher (around double; depending on the plant species, mixture or sampling date) in the Córdoba experimental plot than in the Adamuz plot (Fig. 5). Overall, these differences might be due to the different soil conditions (more fertile and deeper in Córdoba) and slightly better temperature in Córdoba as compared to Adamuz during the growing period. Nevertheless, a minor part of the differences could be due to the delay in the seeding date in the 2014/15 season, or to the advancement of sampling in 2015/16, in Adamuz as compared to Córdoba. It is apparent that the edaphic conditions in each location play a determining role in the aboveground biomass production of a certain CC; thus, in Adamuz the physical-chemical properties of the soil, such as its reduced water storage capacity (restrictive soil depth, light texture and abundance of stone) and fertility, can limit CC biomass production (e.g., Thompson et al., 1991; Belcher et al., 1995; Bingham, 2001).

Our results highlight that CC biomass can be highly variable and site-specific depending on multiple factors (e.g., CC species, been as individual species or mixtures, CC life cycle length, seeding date and rate, CC management, cropping system and climate). As an example, Ruis et al. (2019) in an extensive review that included the 20 most common CC species and mixes in annual cropping systems in semiarid temperate ecoregions (< 750 mm of annual rainfall) recorded CC aboveground biomass yield ranging from 0.2 to 7.4 Mg ha^{-1}, depending on plant species and annual weather conditions. In woody cropping systems in Mediterranean regions, Kazakou et al. (2016) in the inter-rows of a vineyard located in Southern France measured 3.9 and 5.2 Mg ha^{-1} of aboveground biomass in spontaneous CCs and in a sown mix of annual *Medicago* species, respectively; Sulas et al. (2017) in a commercial vineyard located in Sardinia (Italy) measured between 0.6 and 4.7 Mg ha^{-1} of aboveground biomass in a legume CC (burr medic) at maturity, and 1.1–1.2 Mg ha^{-1} in a grass CC (cocksfoot), over three agricultural seasons; and Guzmán et al. (2019) in eight vineyards located in Andalusia (southern Spain) with different CC typologies (e.g., spontaneous vegetation, sown barley) measured aboveground biomass yield ranging from 0.6 to 4.7 Mg ha^{-1}, in a relatively dry year in the region. Torrús-Castillo et al. (2022) in 46 olive groves along Andalusia with temporary spontaneous CCs reported a very high aboveground biomass variability (min 0.2 Mg ha^{-1} yr^{-1}; max 6.7 Mg ha^{-1} yr^{-1}, measured between mid-March and the end of April 2021); Vicente-Vicente et al. (2017) in ten olive orchards located in the provinces of Jaen and Granada (Andalusia), in which spontaneous CCs covered between 40 % and 70 % of the orchard area, measured mean annual CC aboveground biomass that varied from 0.65 to 2.53 Mg ha^{-1}; Repullo-Ruibérriz et al. (2012) in one olive orchard located in the province of Córdoba (Andalusia) measured 2.1, 6.7 and 5.9 Mg ha^{-1} of aboveground biomass in three consecutive years in spontaneous covers of typical weeds of the area; and Tul et al. (2022) in a rainfed olive grove in western Crete measured 8.3 and 2.4 Mg ha^{-1} of aboveground biomass in a sown CC mixture (pea, vetch and oat) and in the spontaneous natural vegetation cover, respectively, in April (peak biomass period for these legumes). Our data of aboveground biomass production (mean values ranging from 1.4 Mg ha^{-1} to 6.9 Mg ha^{-1}) for each type of CC sown in the Córdoba and Adamuz plots were within the most frequent range of values. For the same monospecific CCs as in our study, sown in the lane of commercial olive groves in different locations in Andalusia, Gómez et al. (2018b) measured in four olive orchards in a 2-yr field study a mean annual aboveground biomass production between 1.0 and 4.3 Mg ha^{-1} of *B. rubens*; Rodríguez-Lizana et al. (2018) an average of 6.7 Mg ha^{-1} of *B. distachyon* in one olive orchard over a 4-yr period, and Carpio et al. (2017, 2020) an average in two olive orchards of 0.5 and 1.0 Mg ha^{-1} of *A. arvensis* in two consecutive years. Differences in soil physicochemical properties or CCs growing on degraded soils (compacted, low fertility), seasonal rainfall variability or management would explain the variations in aboveground biomass productivity in these mono-specific CCs and differences relative to our study (Carpio et al., 2017, 2020; Gómez et al., 2018b; Rodríguez-Lizana et al., 2018).

3.3. *Fine root biomass*

The fine root biomass (RB) for the four single-species and the three CC mixtures, at each soil layer and sampling date, is shown in Fig. 6, separately for the experimental plots of Córdoba (Fig. 6a, c) and Adamuz (Fig. 6b, d). In Córdoba, no significant differences were observed in the total weight of fine roots (0–100 cm soil layer) between the three sampling dates (April and May 2015 and April 2016; $p > 0.05$) for any of the mono-specific or multi-specific CCs (Fig. 6a, c). Comparing between species, in general, the total dry weight of fine roots was higher in the plant species with fasciculate roots (i.e. grasses; Br and Bd) than in those with axonomorphic root system (i.e. dicotyledonous; Mt and Aa), with significantly more fine RB in *B. rubens* (mean values between 247 and 310 g m^{-2}) than in *M. truncatula* (188–198 g m^{-2}) and *A. arvensis*

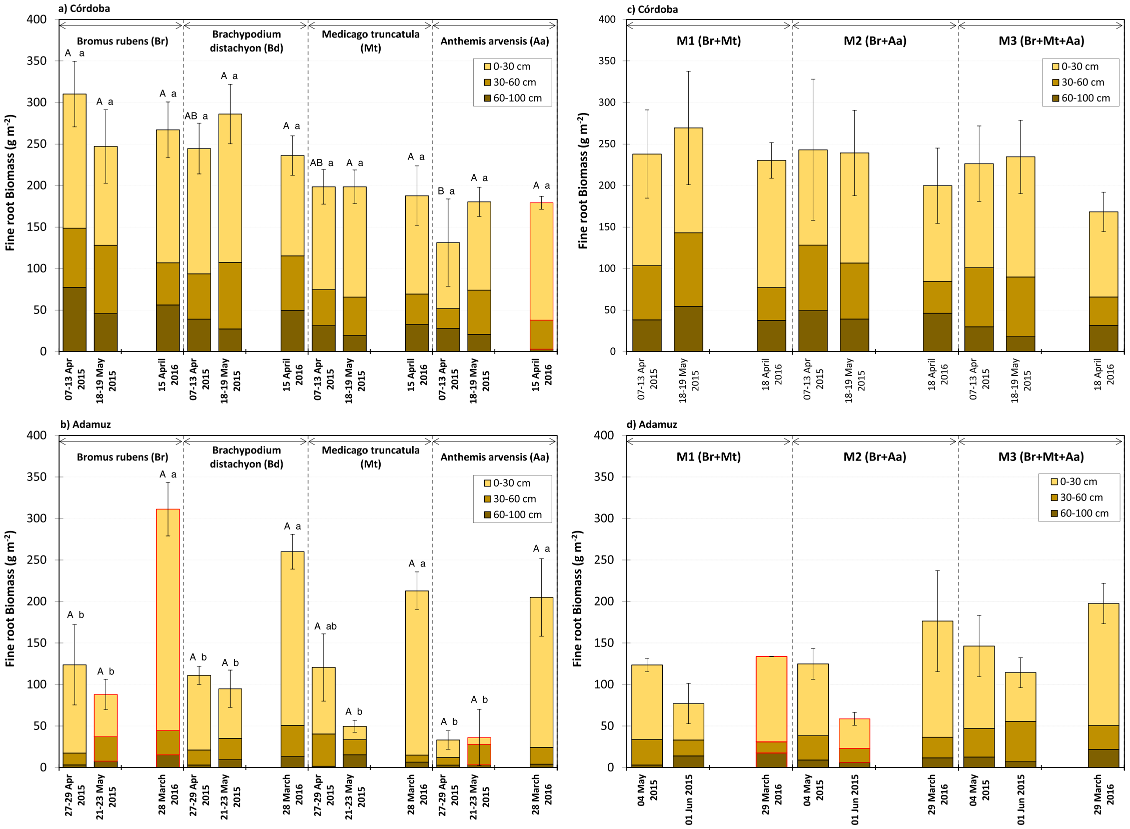

Fig. 6. Mean fine root biomass dry matter (RB; g m^{-2}) of the mono-specific, a) and b) (left), and multi-specific, c) and d) (right), cover crops in the experimental plots of Córdoba, a) and c) (above), and Adamuz, b) and d) (below), at 0–30, 30–60 and 60–100 cm soil depth in the three soil sampling dates carried out in each location. Vertical bars represent the standard errors for the total amount of fine RB (0–100 cm soil depth). Different capital letters indicate significant differences in fine RB (0–100 cm soil depth) between plant species for each sampling date, while different lowercase letters indicate significant differences between the sampling dates for each plant species, according to Tukey's HSD test. There were no statistical differences between the mixtures (M1–M3) for any sampling date, nor between sampling dates for any mixture.

(131–180 g m^{-2}) (Br > Mt = Aa), and in *B. distachyon* (236–286 g m^{-2}) than in Aa (Bd > Aa) ($p < 0.01$) when grouping the three samples (Fig. 6a). The three CC mixtures showed a similar amount of total fine RB (Fig. 6c), without differing from the RB values obtained in the plots of the plant species that make up each mixture. The fine RB was concentrated in the upper soil layers, with 53.4 ± 6.0 %, 58.4 ± 6.3 %, 64.1 ± 2.5 % and 66.2 ± 11.1 % of the weight of fine roots (0–100 cm depth) of Br, Bd, Mt and Aa, respectively, located in the 0–30 cm soil layer averaging the three samplings. This distribution is in line with Fan et al. (2016) in a review including 11 agricultural crops from temperate regions (cereals, legumes and oilseeds), who reported average values between 61 % and 76 % of their root biomass in the uppermost 30 cm soil profile. The mean percentage allocation of fine RB decreased with increasing soil depth, especially in Aa and Mt (11.4 ± 9.8 %, 14.3 ± 4.0 %, 15.5 ± 3.3 % and 21.5 ± 1.9 % of Aa, Mt, Bd and Br, respectively, below 60 cm soil depth) (Fig. 6a), showing a different pattern between grasses and dicots in the distribution of fine RB by soil depth. The distribution pattern of fine RB was similar in the three CC mixtures, with between 53.4 % and 59.3 % concentrated in 0–30 cm soil layer, and between 13.2 % and 19.9 % in soil depth below 60 cm (Fig. 6c).

In the Adamuz plot, the weight of fine roots for each single-species CC was significantly higher (more than double) in the sample of March 28, 2016 (early sowing) than in the samples of April 27–29 and May 21–23, 2015 (late sowing), ($p < 0.001$; Fig. 6b). Thus, total mean fine RB production (up to 100 cm soil depth) varied between 124 and 88 g m^{-2} in Br, 111–95 g m^{-2} in Bd, 120–50 g m^{-2} in Mt, and 33–36 g m^{-2} in Aa in the 2014/15 growing season (April-May, respectively), while it was 311, 260, 213 and 205 g m^{-2} in Br, Bd, Mt and Aa, respectively, in the 2015/16 season, with the same ordering in the amount of fine RB between single-species than that observed in Córdoba (i.e., grasses > dicots). In the CC mixtures, total mean fine RB production was 123–77 g m^{-2} in M1, 125–59 g m^{-2} in M2, and 146–114 g m^{-2} in M3 in the 2014/15 season (May 4–June 1, respectively), and 134, 176 and 198 g m^{-2} in M1, M2 and M3, respectively, in the 2015/16 season (March 29). The much lower amount of fine RB in all the plant species in the 2014/15 season in Adamuz could be due to the late sowing and the delay in seedling emergence until the end of January-beginning of February, which resulted in a shorter time for RB growth as compared to the early sowing in the 2015/16 season (Gregory and Eastham, 1996; Bingham, 2001). The percentage of fine RB in the most superficial soil layers was higher in Adamuz than in Córdoba, with around 95 % of fine RB in single-species and 91 % in CC mixtures distributed in 0–60 cm depth, which was explained by the restrictive soil depth in the Adamuz experimental plot, and between 76 % (Aa) and 86 % (Br) of the fine RB measured at the end of April 2015 and March 2016 distributed in the upper 30-cm of soil (Fig. 6b, d).

Fine root biomass density (RB_d; mg cm^{-3}) for single-species CCs was about three times (Córdoba plot) and between four and nine times (Adamuz plot) higher in the upper 30-cm soil layer than in the next 30–60 cm depth (Fig. 6). In the experimental plot of Córdoba, mean fine RB_d (0–30 cm soil depth) varied between 0.265 mg cm^{-3} (Aa) and 0.539 mg cm^{-3} (Br) including the samplings of mid-April 2015 and 2016 (with similar RB_d on both dates). In the Adamuz plot, the mean fine RB_d was between 0.070 mg cm^{-3} (Aa) and 0.354 mg cm^{-3} (Br) at the end of April 2015, and between 0.602 mg cm^{-3} (Aa) and 0.889 mg cm^{-3} (Br) at the end of March 2016. Mean fine RB_d (0–30 cm soil depth) by CC species was in this order Br ≥ Bd > Mt > Aa, thus suggesting that fibrous-rooted species (i.e., grasses) allocate more biomass to roots than the dicot species in the topsoil layer, similar to that reported by Blanco-Canqui et al. (2020) in a review on root biomass production from the 20 most common CC species in annual cropping systems in temperate regions. Our results also show that a very relevant trait for temporary CCs in Mediterranean conditions might be fine root biomass density, since it reflects the ability of the CC to optimize soil water use in a water-limited environment, as well as its impact on the provision of different ecosystem services, e.g. erosion control or soil carbon storage. It might be particularly relevant to measure this trait in the top 30 cm of the soil, a depth where soil water losses by evaporation are high and the impact of roots on water erosion (sheet and rill erosion) control plays a leading role. It is a trait reflecting the differences among different CC species (e.g., maximum height, branching pattern, life cycle, potential biomass yield), as in the case of Aa, i.e. short cycle and low height and branching (Carpio et al., 2020; Gómez and Soriano, 2020), which resulted in a lower fine RB production, but also the effect of environmental conditions such as soil and climate.

Despite CC root biomass production being a key input for soil ecosystem service delivery (e.g., soil stabilization, soil-erosion control, soil C accumulation, soil health improvement, and others), most CC studies do not consider, or do not measure, root biomass yield when assessing CC benefits (Blanco-Canqui et al., 2020; Ruis et al., 2020). According to the available studies on CC root biomass, the extensive review by Blanco-Canqui et al. (2020), which includes brassicas, grasses, legumes, and other CC groups, reports CC root biomass yields ranging from 0.27 to 5.02 Mg ha^{-1} in the top 30 cm of soil; and, in a later study, Lavergne et al. (2021) in a field experiment pea-based mixtures of up to 12 CC species (grass, legumes, brassicas an others), conducted at three site-years in Quebec (Canada) after harvesting spring barley or spelt, measured mean belowground biomass among all site-years ranging from 0.69 Mg ha^{-1} (1CC or pure pea stand) to 0.92 Mg ha^{-1} (12 CC mixture) in the 0–30 cm soil depth; while Jackson et al. (1996), in a review synthesizing data on root biomass in major terrestrial biomes, calculated a global average of total root biomass of 1.5 Mg ha^{-1} in croplands and 14 Mg ha^{-1} in temperate grasslands. Our study helps to provide additional data on this matter in semiarid conditions. So, mean fine root biomass in the 0–30 cm soil depth ranged between 0.082–1.81 (min–max; Aa) and 0.51–2.67 (Br) Mg ha^{-1} in monospecific CCs and between 0.36–1.40 (M2) and 0.59–1.47 (M3) Mg ha^{-1} in CC mixtures, in the Adamuz experimental plot; and between 0.80–1.06 (Aa) and 1.21–1.79 (Bd) Mg ha^{-1} in monospecific CCs and between 1.15–1.33 (M2) and 1.26–1.53 (M1) Mg ha^{-1} in the mixtures, in Córdoba. In a 3-yr experiment in a Mediterranean vineyard in Sardinia (Italy), Sulas et al. (2017) measured 0.7, 2.9 and 4.9 Mg ha^{-1} of root biomass in a grass CC (cocksfoot) and 0.4–0.5 Mg ha^{-1} in a legume CC (burr medic) at maturity, in the 0–40 cm soil depth; while in a three-year study of irrigated CCs (barley, vetch) during intercropping period of maize in Aranjuez (Central Spain), Gabriel and Quemada (2011) measured mean root biomass yields from 2.00 to 3.42 Mg ha^{-1} in vetch and from 2.07 to 3.18 Mg ha^{-1} in barley, in the 0–40 cm soil depth. Our results are within these ranges of values, but CC species, soil properties, management, cropping system and climate directly interact on fine RB production and distribution, so additional targeted field research should be conducted to achieve a better understanding of fine root biomass production and vertical distribution pattern, and its effects on soil ecosystem services.

3.4. *Fine root length density*

Fig. 7 shows the mean values of fine root length density (RL_d; cm cm^{-3}) for the four single-species and the three CC mixtures in each of the three soil layers (0–30, 30–60 and 60–100 cm) and sampling dates, separately for the experimental plots of Córdoba (Fig. 7a, c) and Adamuz (Fig. 7b, d). Fine RL_d progressively decreased with increasing soil depth in all CCs, single-species and mixtures, with significantly higher RL_d values ($p < 0.001$) in the top 30-cm soil layer at each sampling date in both experimental plots, similar to the distribution pattern shown by fine RB, and it has been widely reported in crop plants and CCs (e.g., Gerwitz and Page, 1974; Hamblin and Tennant, 1987; Fan et al., 2016; Bublitz et al., 2022). Regarding the different measurement dates, fine RL_d values were lower in the second sampling carried out in the 2014/15 season (May 18–19 in Córdoba, and May 21–23-June 1 in Adamuz), when the different plant species had already reached the fully ripe stage and had begun senescence and decay of roots (Fig. 7).

In Córdoba, in the sampling of April 7–13, 2015, mean fine RL_d

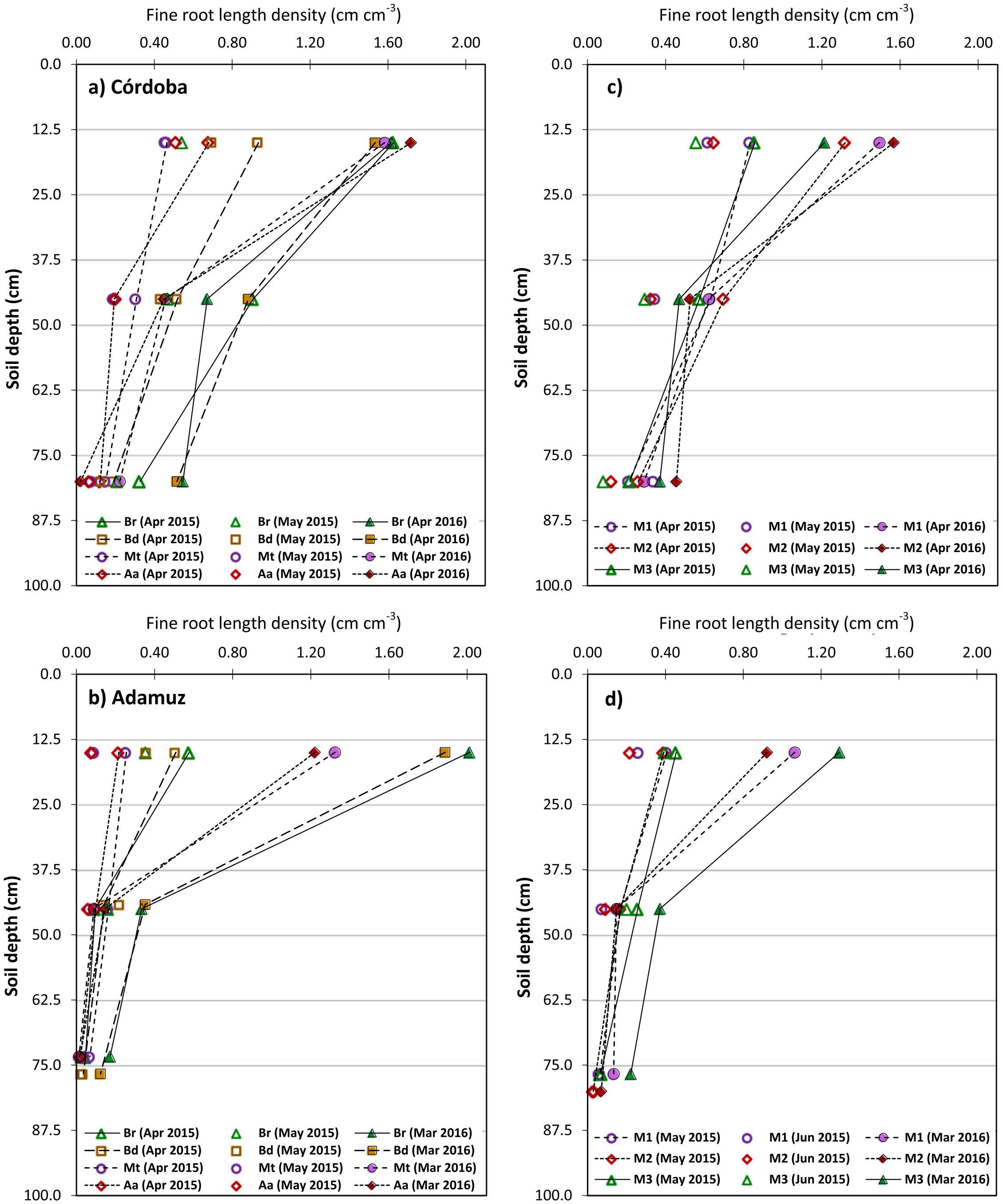

Fig. 7. Mean fine root length density (cm cm^{-3}) of mono-specific, a) and b) (left), and multi-specific, c) and d) (right), cover crops at different soil depth (0–30, 30–60 and 60–100 cm) in the three soil sampling dates carried out in the experimental plots of Córdoba, a) and c) (above), and Adamuz, b) and d) (below).

values in the 0–100 cm soil layer varied significantly between *B. rubens* (0.89 ± 0.50 cm cm^{-3}) and *A. arvensis* and *M. truncatula* (0.31 ± 0.11 and 0.29 ± 0.08 cm cm^{-3}, respectively) (Br > Aa = Mt), with *B. distachyon* taking intermediate values (0.51 ± 0.02 cm cm^{-3}), and showing the three CC mixtures similar and intermediate RL$_d$ values (0.58 ± 0.11 cm cm^{-3}) in accordance with the plant species included in the mixtures. Differences in fine RL$_d$ between grass and dicot species were more accentuated in the top 0–30 cm soil layer: means of 1.63, 0.93, 0.68 and 0.47 cm cm^{-3} in Br, Bd, Aa and Mt, respectively, and with similar-intermediate RL$_d$ values in the three CC mixtures (1.00 ± 0.27 cm cm^{-3}), (Fig. 7a, c). In the May 2015 sampling, fine RL$_d$ values were much lower and without significant differences between mono-specific and/or multi-specific CCs, with mean RL$_d$ between 0.46 and 0.70 cm cm^{-3} in 0–30 cm soil layer and between 0.23 and 0.42 cm cm^{-3} in the 0–100 cm, including mono and mixed CCs. In the sampling in April 15–18, 2016, differences in fine RL$_d$ between single-species or between mixtures were not significant: means of 1.61 ± 0.08 cm cm^{-3} for single-species and 1.43 ± 0.19 cm cm^{-3} for mixtures in the 0–30 cm soil layer, and of 0.80 ± 0.14 cm cm^{-3} for single-species and 0.74 ± 0.08 cm cm^{-3} for mixtures in the 0–100 cm layer. In the deepest soil layer (60–100 cm), fine RL$_d$ values were significantly higher in Br than in Mt and Aa, and in Bd than in Aa ($p < 0.01$), averaging the three samplings, with no differences between the three CC mixtures (Fig. 7a, c).

In the Adamuz plot, the fine RL$_d$ values in the 2014/15 season (late sowing) were significantly lower than those measured in Córdoba

(Fig. 7), similar to what was also observed for the fine RB. In sampling from April 27–29, 2015, the mean fine RL_d in the topsoil layer (0–30 cm) was generally higher in grass species than in dicots: means of 0.58, 0.51, 0.26 and 0.22 cm cm^{-3} in Br, Bd, Mt and Aa, respectively, with significant differences between Br and Mt and Aa (Br > Mt = Aa), and with intermediate and similar RL_d values in the three CC mixtures (0.42 ± 0.03 cm cm^{-3}; May 4, 2015) (Fig. 7b, d). On the second measurement date in the 2014/15 season (at the end of the plants' life cycle), fine RL_d values at 0–30 cm soil depth were significantly higher in *B. rubens* and *B. distachyon* (0.36 cm cm^{-3}) than in *M. truncatula* and *A. arvensis* (0.09 and 0.08 cm cm^{-3}) which completed their life cycle earlier, (Br = Bd > Mt = Aa); while there were no differences in fine RL_d between the CC mixtures (0.29 ± 0.09 cm cm^{-3}). In the soil sampling carried out in the 2015/16 season (early sowing), fine RL_d values in the 0–30 cm soil layer were significantly higher than those measured in the 2014/15 season, but similar to those measured in the Córdoba plot: means of 2.01, 1.89, 1.32 and 1.22 cm cm^{-3} in Br, Bd, Mt and Aa, respectively (March 28, 2016), with similar RL_d values in the three mixtures (1.09 ± 0.19 cm cm^{-3}; March 29, 2016). In the soil layers > 30-cm deep, fine RL_d values were low and lower than those measured in the Córdoba plot, as was also observed for fine RB (Figs. 6 and 7); thus, in the deepest soil layer (> 60 cm), fine RL_d values averaging the three measurement dates were 0.083 ± 0.078, 0.062 ± 0.053, 0.042 ± 0.028 and 0.023 ± 0.004 cm cm^{-3} in Br, Bd, Mt and Aa, respectively, and 0.084 ± 0.038 cm cm^{-3} including the three CC mixtures, for a maximum soil depth of 0.80 ± 0.03 m in the single-species plot and 0.91 ± 0.10 m in the mixed-species plot (Fig. 7b, d).

In studies with crops, typical values of fine RL_d in the upper 0.10 m of soil are about 5–10 cm cm^{-3} in temperate cereal crops, and 1–2 cm cm^{-3} in other crops, with the roots distributed in the soil such that RL_d decreases exponentially with depth (Gregory, 2006). In two-year CC rotation in the Northeast USA, Gardner and Sarrantonio (2012) measured in red clover and winter rye of different ages nearly 50 % of the fine RL_d in the upper 5-cm for all roots, with no significant differences between species and ages, and in two-year field study of CCs from three different families in Hennef (Germany), Bublitz et al. (2022) reported mean cumulative RL of 86.6 %, 67.3 % and 81.2 % for grasses, brassicas and legumes, respectively, in the topsoil 0–30 cm, with the greatest mean values of RL_d of 7.2, 5.0 and 2.4 cm cm^{-3} (0–30 cm depth), and 2.5, 2.3 and 0.9 cm cm^{-3} (0–100 cm depth), respectively. In our study, in the rainfed Mediterranean conditions of southern Spain, the RL_d data were in the range of the values reported by Gregory (2006) but lower than RL_d values measured by Bublitz et al. (2022) in grasses and legumes. Fine roots proliferation and their distribution in the soil with depth are affected by both genetic (e.g., plant functional types) and environmental factors (soil type, climate), (e.g., Gregory, 2006; Freschet et al., 2017); in addition, locally, the spatial and temporal heterogeneity in soil physico-chemical and biological properties and in soil-based resources (e.g., availability of water and nutrients to plants, soil bulk density) is one of the major causes of variation in root proliferation and in root distribution patterns in the soil profile (Hodge, 2010; Bengough et al., 2011). In the case of the Mediterranean region, roots proliferation and distribution will frequently be determined by the soil profile rewetting by rainfall, which varies with both the site and season/year and throughout the plants' life cycles (Padilla et al., 2015).

3.5. *Soil water use by cover crops*

Soil water use (or crop evapotranspiration, ET) by monospecific (*B. rubens* and *M. truncatula*) and multispecific (Br + Mt and Br + Mt + Aa) cover crops in the Córdoba experimental plot was similar for all CCs, and without significant differences between the two growing seasons. Cumulative ET (mean±SD) during the two CC growing seasons was 321 ± 4 mm (2014/15 season) and 310 ± 12 mm (2015/16 season), and the corresponding cumulative ETo was 367 and 343 mm (Table 3). No water stress symptoms were observed in any of the CCs in either of the two growing seasons, with accumulated rainfall from the start of the agricultural year (September 1) to full seeds maturity (early May) of 472 and 415 mm in the 2014/15 and 2015/16 seasons (Table 3).

Table 3
Water use (ET, mm) by mono- (Br; Mt) and multi-specific (Br + Mt; Br + Mt + Aa) cover crops (average ± SD), and accumulated rainfall and ET_0 (mm) during the 2014/15 and 2015/16 cover crop seasons, in Córdoba.

Cover crop season: measurement period	Cover crop ET (mm)				Rainfall (mm)	ET_0 (mm)
	Br[i]	Mt	Br + Mt	Br + Mt + Aa		
2014/15: November 19, 2014 to May 5, 2015	316 ± 10	319 ± 10	321 ± 4	326 ± 16	205 (472)[ii]	367
2015/16: November 30, 2015 to May 3, 2016	297 ± 6	322 ± 26	303 ± 28	319 ± 12	259 (415)[ii]	343

[i] Br = *B. rubens*; Mt = *M. truncatula*; Aa = *A. arvensis*.
[ii] accumulated precipitation since the beginning of the agricultural season (September 1) to April 30.

Cover crop water uptake was mostly concentrated in the top 0.90-m soil layer (over 90 % of total ET) (Fig. 8), since below 0.90-m depth the fine roots length density was negligible (Fig. 7a, c). The estimation of soil-water extraction by roots below 0.90 m soil depth was 21 mm and 35 mm in the 2014/15 and 2015/16 seasons, respectively, with no significant differences between the different CCs (monospecific and mixtures), and with SWC depletion below 90-cm depth that occurred in the last period of the cover crops cycle (end of March–April) (Fig. 8). The mean volumetric soil moisture (m^3 m^{-3}) at the end of CC cycles (early May) was 9.7 % (0–90 cm depth) and 15.3 % (90–150 cm depth) in the 2014/15 season, and 14.2 % (0–90 cm) and 15.3 % (90–150 cm depth) in the 2015/16 season, with the differences in soil moisture in the first 0–90 cm depth between both seasons mainly due to the more rain in 2016 than in 2015 in the last three weeks of April.

The evolution in volumetric SWC throughout the CC growth cycles in the two seasons in this study did not indicate that the soil water contents or CC water use was different for single-species or for mixtures of these species, which coincides with the results obtained by Nielsen et al. (2015) in a semi-arid environment in the Midwest USA when comparing SWC and crop water use between CCs grown in single-species and in a 10-species mixture. Soil water use by CCs was concentrated in the last third of their growing cycles, with between 55 % and 61 % of cover crop ET consumed from mid-March to the end of April, when the atmospheric evaporative demand increased (mean ETo of 3.6 mm day^{-1}). This fact highlights the key value of an early sowing date and the search for short-cycle cover crop species, to avoid competition for soil water with woody crops at a time of high evaporative demand and low rainfall in the Mediterranean region (Gómez and Soriano, 2020).

4. Conclusions

Our study showed that thermal time to achieve 30 % ground cover and seed maturity and fine RB, or RL, in the top 0–30 cm appeared as three key traits to evaluate the suitability of autochthonous species as temporary CCs for Mediterranean woody crops in the four species evaluated *B. rubens* (Br), *B. distachyon* (Bd), *M. truncatula* (Mt), and *A. arvensis* (Aa). The first trait because its relationship with protection against soil erosion during the erosive period, and the second trait to ensure seed production before the onset of competition for soil water. The third one reflects the ability of the CCs to optimize water use in a water-limited environment, and their impact on the provision of different ecosystem services, e.g., erosion control or soil carbon storage. Early seeding (no later than mid-October) is critical to exploit these

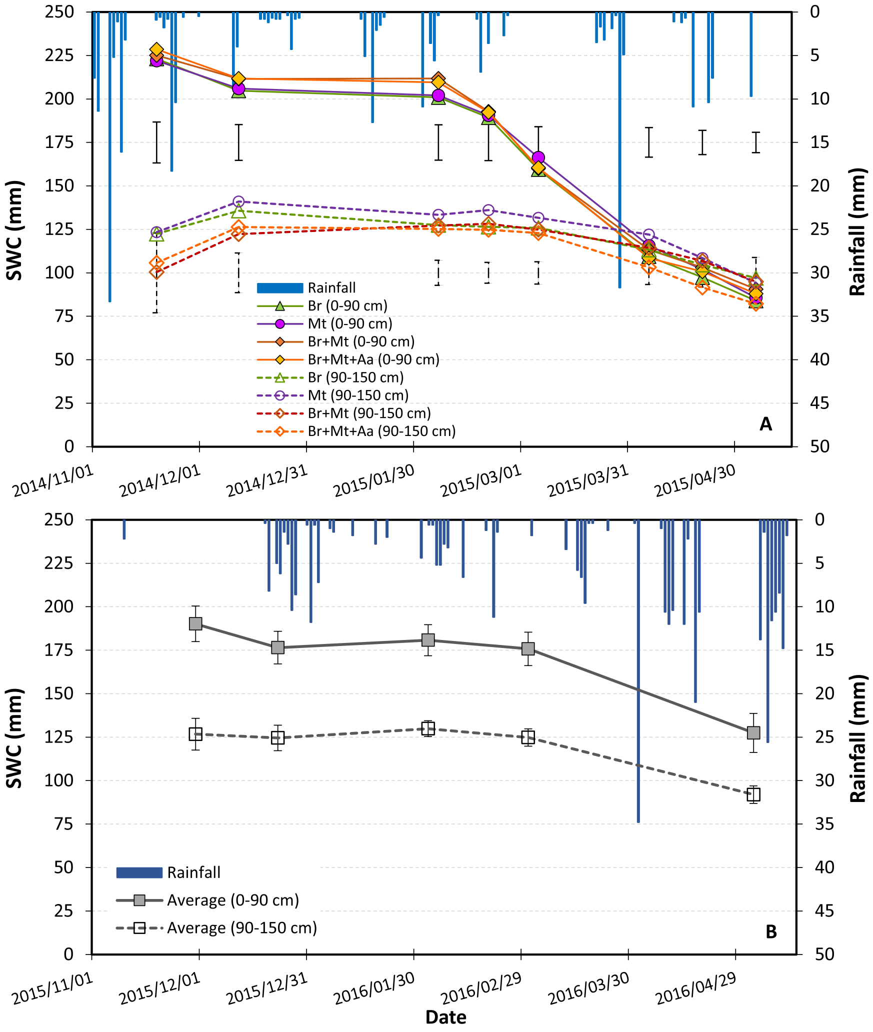

Fig. 8. Evolution of soil water content (SWC, mm; at 0–90 cm and 90–150 cm soil depth), and daily rainfall (mm) in the experimental plot of Córdoba from seedling pre-emergence to post-maturity of seeds during the 2014/15 and 2015/16 cover crop seasons under rainfed conditions. A) Vertical bars represent the mean standard errors (SE) of all mono- (Br; Mt) and multi-specific (Br + Mt; Br + Mt + Aa) cover crops; B) SWC and SE values are average values over all cover crops. Br = *B. rubens*, Mt = *M. truncatula*, and Aa = *A. arvensis*.

traits into effective CCs under Mediterranean conditions. They can be fully exploited within the regions using the available phenologic models developed for the tested species, based on thermal time, which predicted properly their development under field conditions in our experiment. Ground cover evolution and biomass production were also greatly affected by edaphic-climatic conditions. Grasses species (Br, Bd) and legume (Mt) presented the maximum GC (above 90 %), as compared to the composite (Aa, around 80 %). Aboveground biomass was higher values in the more fertile location, Cordoba, 300–700 g m^{-2}, as compared to Adamuz, 150–350 g m^{-2}. Grasses (Br, Bd) and legume (Mt) presented a higher aboveground biomass in both sites as compared to Aa, 600 vs. 300 g m^{-2} and 300 vs. 200 g m^{-2} for Córdoba and Adamuz respectively, although we observed no differences among mixtures within each of the two locations. Fine root biomass followed and fine root density resembles somehow those of aboveground biomass, albeit with a higher variability. Overall, fine RB (0–100 cm soil depth) was in the range of 130–310 and 35–310 g m^{-2} for Córdoba and Adamuz respectively, with around 60–75 % of this RB concentrated in the top 30-cm of the soil. We observed no significant differences in soil water uptake among the different among monospecific CCs or mixes in Córdoba, with ET values during the 2014/15 and 2015/16 growing seasons in the 325–300 mm range.

CRediT authorship contribution statement

José Alfonso Gómez and María-Auxiliadora Soriano conceived the idea of the presented study. Field measurements were carried out mostly by José Manuel Cabezas with support from María-Auxiliadora Soriano. The manuscript was written by María-Auxiliadora Soriano with help from José Alfonso Gómez. All authors discussed the methods and results and contributed to the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2022.126723.

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