

## Research Article

# *Brachypodium hybridum* Plant Cover Improves Water Infiltration in Mediterranean Crop Soils

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Crop plant cover may be an appropriate solution to avoid soil losses by erosion under Mediterranean climate, where traditional tillage aims to improve rainfall water infiltration, and direct evaporation from bare soil is avoided because plant water transpiration is a major limiting factor for non-irrigated crop productivity. Twelve crop lines of the three *Brachypodium distachyon* complex species with different chromosome number (*B. distachyon*, 2n=10; *B. stacei*, 2n=20; and *B. hybridum*, 2n=30) were grown in a field trial to assess water infiltrability and hydraulic conductivity across crop topsoils considering two different plant cover densities (low cover = 150 plants/m<sup>2</sup>; regular cover = 450 plants/m<sup>2</sup>), and in a control no-tillage bare soil. Results showed that superficial hydraulic conductivity was significantly higher in *Brachypodium*-covered soils with regular plant density (3.254 ± 0.710 cm·h<sup>-1</sup>) than in the no-tillage bare soil (1.965 ± 0.711 cm·h<sup>-1</sup>). On an extreme ranking basis, yields were 1.89 < k(h<sub>0</sub>) cm/h < 27.12 under covered soils, and 0.679 < k(h<sub>0</sub>) < 4.330 in the no-tillage bare soil. In conclusion, *B. distachyon* ecotypes can protect soil from being eroded and improve water soil infiltration. The adaptation of *B. hybridum* to Mediterranean environments represents an interesting alternative as cover crop for typical woody agricultural plantations in Mediterranean soil such as olive groves, vineyards, and dry-fruit cropland.

**Keywords:** *Brachypodium distachyon*; *B. stacei*; *B. hybridum*; Mediterranean agriculture; No-tillage; Plant biomass partition; Soil properties; Soil water content; Topsoil conservation; Unsaturated hydraulic conductivity

**Introduction**

Soil water infiltrability across soil surface, sorptivity, and diffusivity are relevant physical properties of top soils. Unsaturated water flow through topsoil is involved in water infiltration under light rainfall conditions, and unsaturated hydraulic conductivity [k(h<sub>0</sub>)] at suction (h<sub>0</sub>>0) strongly depends on pore geometry, connectivity, and soil volumetric water content (θ<sub>v</sub>). As a result, drying soil primarily replaces water with air in soil pores. Soils in native woods typically show high k(h<sub>0</sub>) because of the presence of soil organic matter, the soil-root interface influence, and the existence of preferential flow channels provided by decayed roots [1].

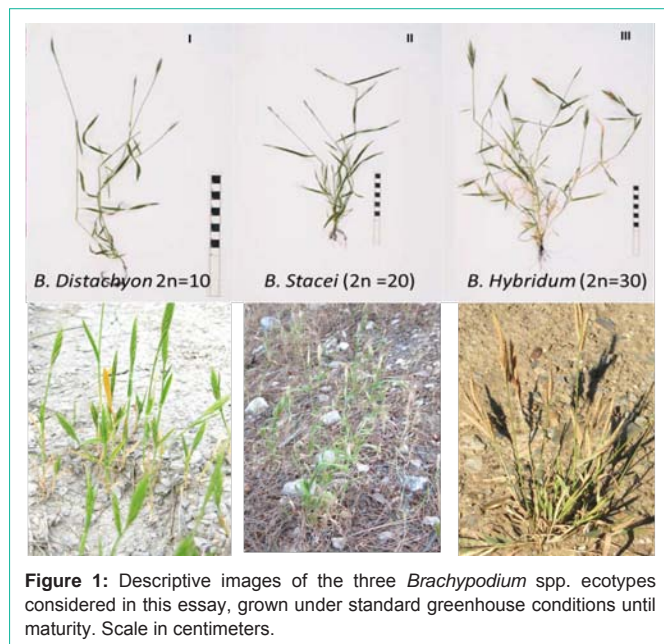
Choosing between conventional tillage and no-tillage agricultural management system is still a dilemma in Mediterranean soils [2]. Olive groves began to be cultivated on no-tillage bare soils from 1979 [3], but other studies have shown that using plant cover was the most effective way to fight against soil erosion and to increase water availability by infiltration during rainy seasons [4]. Spontaneous vegetation associated with a main crop may however turn to be a drawback for the crop yield because of its water consumption, particularly within Mediterranean climate [5].

Consequently, crop plant cover complemented with *Brachypodium distachyon* (L.) P. Beauv., or any of its recently segregated species, may be a good alternative to improve topsoil water infiltration in comparison to plant cover composed by *Secale cereale* (L.) M. Bireb, *Hordeum vulgare* (L.), or spontaneous native

vegetation. In fact, some *B. distachyon* complex segregated species have been domesticated over several generations, and commercialized for soil and water conservation purposes in Spanish olive groves [6].

These *Brachypodium* species have also been adopted as model plants to assess the behaviour of temperate cereals, forage grasses, and biofuel grass crops [7,8]. This group includes interesting species that can be grown between crop lines, and may therefore be selected for: (1) short-life cycle growing, as short as 6 weeks [7,9]; (2) self-fertility, ensuring the generation of pure inbred lines within two generation cycles [10]; (3) high germination rate under wild and controlled greenhouse conditions [9,10]; (4) its phylogenetic relationship with economically important *Poaceae* species, such as *Triticum* spp., *Hordeum* spp., and *Oryza sativa*, a tropical cereal with a fully sequenced genome [11-13]; (5) its species-specific chromosome number, which correlates with altitude and latitude [13], resulting that *B. hybridum* 2n=30 ecotypes are frequent in lowland and coastal areas, and in intermediate altitudinal areas, and do not require vernalisation for flowering [10,13-16]; (6) its high root tensile strength compared to other grasses, shrubs, and small trees (*Brachypodium* species ranked second amongst representative Mediterranean plant species of these groups, as reported in Baets et al. [17]).

Certain relationships link the tension infiltrometer, pressure, and soil core estimates of saturated soil k(h<sub>0</sub>) [18]. Nonetheless, no references have been found to date addressing *Brachypodium* species plant cover and unsaturated soil k(h<sub>0</sub>). This research trial aimed to assess several *Brachypodium* species as plant cover on soils



**Figure 1:** Descriptive images of the three *Brachypodium* spp. ecotypes considered in this essay, grown under standard greenhouse conditions until maturity. Scale in centimeters.

of Mediterranean woody crops interlines, such as olive groves and vineyards, in terms of soil water infiltrability improvement under unsaturated suction conditions.

## Materials and Methods

### Plant material and layout

Twelve *Brachypodium distachyon*, *B. stacei* and *B. hybridum* lines (four lines per species) were selected to be cultivated along 3 years in soils classified in the large group of the *Haploxera* [19,20], located at “Finca la Canaleja”, an experimental field that belongs to the “Instituto Nacional de Investigaciones Agrarias” (INIA), in central Spain near Madrid (latitude = 40° 30' 44" N; longitude = 03° 08' 52" W; altitude=601 m). This area is subjected to a Mesomediterranean type of climate, with average annual atmospheric precipitation of 438 mm, and an average annual temperature of 13.5°C. The corresponding pedo-climate has a *Xeric* soil moisture regime and a *Mesic* soil temperature regime [21].

These *Brachypodium* species have recently been described as separate species [22,23], namely *Brachypodium distachyon* diploid, 2n=10 (x=5), *Brachypodium stacei*, diploid, 2n=20 (x=10), and *Brachypodium hybridum*, allotetraploid 2n=30 (x=5+10). This group of species was selected because Spain is rich in wild populations [13,15,23,24] that display wide variability in seed characteristics and weight [7] according to environmental diversity. *Brachypodium* species generally show different chromosome base numbers (i.e. diploidy; Catalán et al. [13]), and *B. stacei* and *B. hybridum* tend to be larger than *B. distachyon* [14,23,25,26]. *B. hybridum* does not need vernalisation to flower, and it is therefore able to grow under wider environmental conditions than its diploid *B. distachyon* progenitor (Figure 1).

The 12 *Brachypodium* lines selected to be sown in this trial were the following: (a) four *B. distachyon* (2n=10) lines, labeled D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub>, and selected from central Spanish locations at an altitude of 830-1200 m (Madrid, Guadalajara and Cuenca); (b) two *B. stacei*

(2n=20) lines selected in peripheral Spanish locations at altitudes of 42-949 m, labeled T<sub>3</sub> and T<sub>4</sub> (Valencia and Albacete); (c) two other *B. stacei* populations from Iran, labeled A<sub>1</sub> and A<sub>2</sub>, which were used as control foreign populations; and (d) four *B. hybridum* (2n=30) lines, labeled H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub> and H<sub>4</sub>, selected from locations of peripheral Spain at altitudes of 23-759 m (Córdoba, Jaen and Albacete, and between Seville and Huelva).

The crop trial layout consisted of 12 random small rectangular plots (18 x 22 m<sup>2</sup>) per *Brachypodium* species and each of the following crop systems: (1) two plant cover crop systems (CC by randomizing the planting of 12 species of the *Brachypodium distachyon* complex in the plot): one considering a low plant density of 150 (±50%) plants/m<sup>2</sup> (LOW CC); and another one considering a regular plant density of 450 (±50%) plants/m<sup>2</sup> (REG CC); (2) a no-tillage system on bare soil (NT). The LOW CC system was obtained from a sowing seed density of 400 seeds/m<sup>2</sup>, whereas REG CC was set corresponding to a 1400 seeds/m<sup>2</sup> seed density. Seeds were manually sown. Three increasing doses of nitrogen fertiliser [NH<sub>4</sub>(NO<sub>3</sub>)] were added in the seeding year (0, 100, 200 kg ha<sup>-1</sup>) after emergence to favour plant establishment and proper growth. Plant cover was evaluated counting the number of plant individuals inside a sampling 12x12 cm<sup>2</sup> small square.

Leaf Weight Ratio (LWR, g g<sup>-1</sup>) and Specific Root Length (SRL, cm mg<sup>-1</sup>) were used to discriminate differences between plant/root growth ratios of the assessed *Brachypodium* cytotype species [27,28]. Plant samples were gently washed, and their roots, leaves and stems were separately weighed after being dried in an oven at 75°C until weight became constant (typically 3 days after placing samples in the oven).

### Soil analyses and infiltrability determinations

Twenty-four cylindrical soil samples (10 cm deep; eight corresponding to each NT, LOW CC and REG CC management systems) were air-dried, and standard procedures for soil analyses [29,30] were carried out to determine granulometric fractions (sand between 2 mm and 60 μm, silt fraction between 20-60 μm, fine silt fraction between 2-20 μm, and clay fraction < 2 μm) by the pipette method.

Soil bulk density (BD, kg m<sup>-3</sup>) and natural water content (NWC, %) were determined by standard soil analysis methods [31]. BD was measured by the core method [32] after collecting soil samples with 10-cm-high stainless steel cylinders that contained a total volume of 340 cm<sup>3</sup>. Cylinders were pushed into soil with both ends open using a hydraulic device [29]. Volumetric NWC was determined by drying samples at 105°C until constant weight [33]. Soil Organic Matter (SOM) was measured by wet oxidation following the Walkley-Black method [34]. Electrical Conductivity (ECe, dS/m at 25°C) was determined in aqueous saturation extract [35]. Soil analysis results are shown in Table 1.

After infiltration through soil surface, water vertically flows from surface soil to the bottom according to its vertical k(h<sub>0</sub>). The flow in unsaturated soil is affected by approximately constant gravity and variable suction (h<sub>0</sub><0). The assay to determine infiltrability and vertical unsaturated k(h<sub>0</sub>) was carried out using a Decagon minidisk infiltrometer with a 4.5-cm diameter disc, and by exerting a tension of h<sub>0</sub>=2 cm. The parameters that were obtained by this infiltrometer

**Table 1:** Statistical summary of the general descriptive soil properties of the three sets of eight plots that correspond to the following management systems: no-tillage bare soil (NT), low plant density cover (LOW CC) and regular plant density covered (REG CC).

Soil parameter	Treatment	Min	Max	Mean <sup>a</sup>	Median	SD
SOM (%)	NT	0.18	0.69	0.52a	0.58	0.18
	LOW CC	0.38	0.87	0.52a	0.72	0.15
	REG CC	0.39	0.78	0.55a	0.55	0.15
EC <sub>e</sub> (dS/m at 25°C)	NT	0.121	0.183	0.152a	0.153	0.021
	LOW CC	0.129	0.155	0.149a	0.152	0.008
	REG CC	0.135	0.158	0.148a	0.149	0.010
Clay < 2 μm (%)	NT	12.2	16.4	13.9a	13.9	1.4
	LOW CC	12.6	17.1	13.7a	13.3	1.4
	REG CC	11.2	13.6	12.3b	12.2	0.7
Fine silt 2 - 20 μm (%)	NT	8.7	12.7	10.8a	11.5	1.7
	LOW CC	8.4	11.9	09.6b	9.1	1.3
	REG CC	8.0	10.4	08.8b	8.6	0.7
Coarse silt 20 - 60 μm (%)	NT	12.7	17.4	14.6a	14.3	1.5
	LOW CC	12.8	16.5	13.9ab	13.7	1.1
	REG CC	10.0	13.0	11.7b	11.5	1.0
Sand 0.060 - 2 mm (%)	NT	53.6	64.9	60.6b	60.7	4.1
	LOW CC	53.4	66.6	62.7b	63.8	3.8
	REG CC	64.4	69.5	67.2a	67.4	1.7
NWC (%)	NT	2.21	8.06	5.74a	6.98	2.53
	LOW CC	7.13	8.41	7.85b	7.94	0.52
	REG CC	7.06	8.45	7.54b	7.41	0.48
BD (kg·m <sup>-3</sup> )	NT	1405	1562	1484c	1477	50
	LOW CC	1593	1798	1654b	1667	35
	REG CC	1545	1867	1711a	1687	97

<sup>a</sup>Significant differences at the 95% level of significance (LSD test) are labeled by different letters; SD = standard deviation.

device were: (1) Infiltration Velocity (InfVel), that is, infiltration rate or infiltrability ( $i$ , mm·s<sup>-1</sup>); and (2) Accumulative Infiltration (InfAcum), i.e. infiltration ( $I$ , litre·m<sup>-2</sup> = mm), as determined by the following formula:

$$i = dI/dt = V d\theta_v/dt$$

Where  $t$  is time and  $\theta_v$  is the volumetric water content (m<sup>3</sup>·m<sup>-3</sup>) in the soil profile affected by water infiltration, with a soil volume per m<sup>2</sup> of soil surface equal to  $V$  (mm).

Infiltration ( $I$ , mm) can be estimated in dry soils by the equation proposed by Zhang [36], which uses the two first terms of the equation addressed by Philip [37], that is:

$$I = S t^{1/2} + Bt$$

Where  $S$  is called sorptivity, a parameter that is approximately equal to cumulative infiltration during the first unit of time, i.e. a measure for the capacity of soil to absorb water;  $B$  is a parameter related to the unsaturated hydraulic conductivity [ $k(h_0)$ ] at suction  $h_0$  that is estimated by the following equation:

$$k(h_0) = A/B$$

Where  $A$  is the a-dimensionless van Genuchten parameter [38,39], which depends on soil type, suction value, and the diameter of the infiltrometer disc. Considering a sandy loam soil texture such as the one found in the experimental field, suction  $h_0=2$  cm, and the diameter of the infiltrometer disc (4.5 cm), the van Genuchten parameter value resulted  $A = 4.24$ .

Unsaturated  $k(h_0)$  was estimated considering the above formula [36] by computing the measured InfAcum vs. the square root of time, and by fitting the results with the dimensional formula L·T<sup>-1</sup>, which conveniently resulted in  $k(h_0)$  in units of mm·s<sup>-1</sup> or cm·h<sup>-1</sup> (1 mm·s<sup>-1</sup> = 360 cm·h<sup>-1</sup>).

### Statistical and geostatistical analysis

A standard statistical analysis (mean, median, standard deviation, etc.) was carried out to describe the different analysed parameters. Differences between experimental groups were first determined by analysis of variance (ANOVA). However, these statistical analyses ignore spatial variability, which may be the result of differences among plots characteristics widespread the experimental field; for example, not very big differences in SOM or clay content may significantly affect unsaturated  $k(h_0)$  value.

Simple geostatistical techniques based on semi-variograms determination have increasingly been used to analyze the spatial pattern of soil variables [40-42]. Experimental semi-variograms were calculated to analyze spatial autocorrelation of edaphic variables, and to determine the spatial dependence range [43,44]. The spatial correlation range should be interpreted as the separation distance beyond which observations are not spatially dependent [41,42,45]. Normally, a wide range expresses a major area of influence, which is also attributed to intrinsic properties [46,47]. Unsaturated soil  $k(h_0)$  was mapped by Ordinary Kriging (OK) based on the parameters that derived from the spherical model fitted to semi-variance data. The prediction accuracy of the unsaturated soil  $k(h_0)$  map was evaluated by the cross-validation technique, which removes each data location, one at a time, and predicts the associated data value. Cross-validation analysis served to compare measured and predicted values. Spatial errors are so determined by making a comparison between the experimentally measured  $k(h_0)$  values and the estimated  $k(h_0)$  values obtained from the spatial model. These errors reflect unsaturated  $k(h_0)$  variability among field trial plots, as indicated by standard deviations of original data, and by the uncertainty that is inherent to interpolating from a widely dispersed site [48-50]. As a result, kriging-estimated errors are considered a covariate to avoid the variability attributed to environmental factors that affected experimental field plots. The residuals of Kriging analyses, as a random component and a new independent variable, provide short-scale information of the variation of unsaturated soil  $k(h_0)$ . These differences in the kriged values of unsaturated  $k(h_0)$  were therefore used as a covariate in a new ANOVA performance.

## Results

### Soil characterization

Topsoils of field plots were not homogeneous (Table 1). At the 95% level of probability confidence, the Shapiro-Wilks test nevertheless showed no significant differences in Soil Organic Matter (SOM) and salinity, estimated by the EC of the saturated



**Table 2:** Standard statistical summary of the cumulative infiltration of water in soil (I) and unsaturated hydraulic conductivity [ $k(h_0)$ ] of topsoil in the three sets of eight plots that correspond to the following management systems: no-tillage bare soil (NT), low plant density cover (LOW CC) and regular plant density cover (REG CC).

Soil parameter	Treatment	Min	Max	Mean*	Median	SD
I (cm)	NT	0.169	0.602	0.328b	0.283	0.145
	LOW CC	0.175	0.961	0.440ba	0.583	0.245
	REG CC	0.316	1.130	0.726a	0.650	0.273
$k(h_0)$ ( $\text{cm}\cdot\text{h}^{-1}$ )	NT	0.679	2.906	1.965b	2.080	0.711
	LOW CC	0.849	3.311	2.303b	2.122	0.831
	REG CC	2.292	4.330	3.254a	3.165	0.710

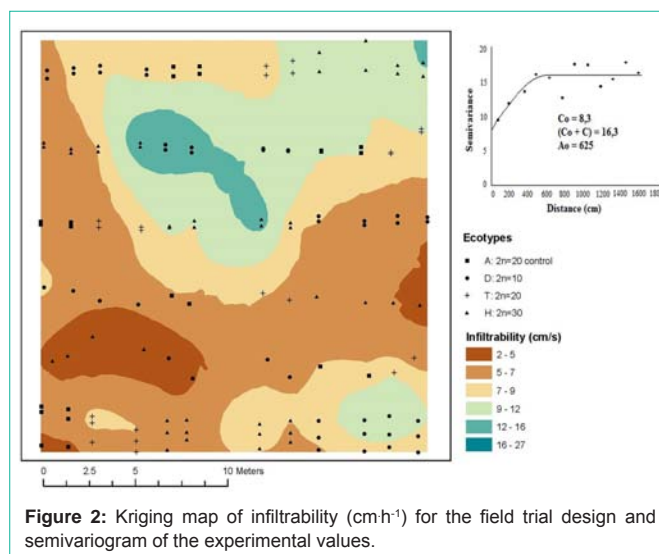
\*Significant differences at the 95% level of significance (LSD test) are labeled by different letters; SD = standard deviation.

**Table 3:** Statistics of the measured vs. estimated values of field unsaturated hydraulic conductivity,  $k(h_0)$  in  $\text{cm}\cdot\text{h}^{-1}$ , through top soils covered with 12 lines of different *Brachypodium distachyon*, *B. stacei* and *B. hybridum* cover crops.

Brachypodium cytotypes			Measured values		Estimated values		Spatial errors	
PI	Family	n	mean	SD	mean	SD	mean	SD
2n = 10	D <sub>4</sub>	11	2.578	0.664	2.528	0.631	0.050	0.744
	D <sub>3</sub>	11	2.361	1.133	2.629	0.801	-0.268	0.997
	D <sub>2</sub>	11	2.692	0.989	2.583	0.673	0.109	1.093
	D <sub>1</sub>	11	2.716	1.233	2.867	0.785	-0.151	1.132
2n = 20	A <sub>2</sub>	11	2.792	1.093	2.577	0.722	0.215	0.537
	A <sub>1</sub>	11	2.228	0.955	2.497	0.964	-0.254	0.732
	T <sub>2</sub>	11	3.225	1.898	2.961	0.946	0.265	1.461
	T <sub>1</sub>	11	3.047	2.555	3.149	1.240	-0.102	2.394
2n = 30	H <sub>4</sub>	11	3.077	1.516	2.797	1.180	0.280	1.550
	H <sub>3</sub>	11	2.870	1.511	3.020	1.083	-0.150	1.235
	H <sub>2</sub>	11	3.379	1.236	2.904	0.934	0.475	0.902
	H <sub>1</sub>	11	2.468	0.988	2.900	0.829	-0.432	0.777
<b>Total</b>		<b>132</b>	<b>2.794</b>	<b>1.382</b>	<b>2.795</b>	<b>0.789</b>	<b>-0.001</b>	<b>1.211</b>

extract, among the soils of these plots, located low terrace of River Henares; although significant differences were observed in particle size and water content. These differences were not however enough to explain the differences that were found in Bulk Density (BD) figures among the three sets of field plots which soil cover properties resulted different. Therefore, the treatment of these soils may have been responsible for an increased BD in the no-tillage bare soils (NT) compared with the low plant cover (LOW CC) and regular plant cover crops (REG CC). In summary, these soils are classified in the large group of Haploxeralfs.

The addressed differences in particle size distribution (Table 1) among these topsoils were consistent with continental heteromeric sediments from fluvial deposition, and also with the residual accumulation of sand in topsoil because of the natural pedogenesis of their A-Bt-Ck soil horizons sequence. The increase in BD from the NT to LOW CC and REG CC plots was in accordance with both the increase in sand content and the decrease in clay and silt contents. However, soil compaction maybe partially explained by the tension of water that is exerted during soil desiccation, promoted by the presence of *Brachypodium* plants density and their corresponding



**Figure 2:** Kriging map of infiltrability ( $\text{cm}\cdot\text{h}^{-1}$ ) for the field trial design and semivariogram of the experimental values.

water consumption.

### Soil hydraulic conductivity and infiltration

Cumulative infiltration of water in soil (I) and unsaturated topsoil  $k(h_0)$  significantly increased with *Brachypodium* plant cover (Table 2), which did not agree with the previously reported increased soil compaction provided by BD figures (Table 1) on the basis of the 24 field trial samples that were analysed (NT, LOW CC, REG CC). This surely occurred by the influence of secondary permeability, caused by adsorbed water on vertical surfaces of small root channels and dependant on vegetation cover density and developed root systems.

Table 3 shows the relations obtained by classical statistical and spatial approach analyses for cumulative water infiltration (I) in soil, and unsaturated  $k(h_0)$ , across the topsoils of the experimental field plots, and on the basis of 132 determinations equally distributed by the designed treatments (LOW CC, REG CC; data not shown). As a result, infiltration (I) ranged from 0.47 to 5.03 cm showing no significant differences among the 12 lines of the three *Brachypodium* species by the classical ANOVA test. Unsaturated  $k(h_0)$  ranged from 1.89 to 27.1  $\text{cm}\cdot\text{h}^{-1}$ ; extreme values were identified close to the coloured zones on the map (Figure 2), and significant differences among the three *Brachypodium* species within CC treatments (LOW CC, REG CC) were addressed by classical ANOVA (Table 4).

Ranking differences were observed for I and  $k(h_0)$  among different species and management treatments (NT, LOW CC, REG CC), which can be partially explained by the influence of soil granulometric particle distributions, the presence of crop cover, and the soil compaction effect on (I) and  $k(h_0)$ . The spatial pattern in the geostatistical analysis was described in dissimilarity terms of the observed data according to the distance in between. The semivariogram (Figure 2) showed the degree of spatial continuity for  $k(h_0)$  across spatial locations, and the spatial dependence given by the scale range. This scale range displayed a spatial influence at a 6.25 m distance, which considered the distance of spatial dependence between field measurements. The spatial variability of water infiltration rate through topsoil surface could be affected by intrinsic traits of soils, such as structure, texture, and SOM content; as well as by extrinsic ones, like agricultural practices (e.g. tillage), seed density, or type of the species used as plant cover.

**Table 4:** Analysis of variance for unsaturated soil hydraulic conductivity ( $k(h_0)$ ,  $\text{cm}\cdot\text{h}^{-1}$ ) by ploidy,  $\text{NH}_4(\text{NO}_3)$  fertilization, and plant density as sources of variation. Kriging residuals of the plots were used as a covariate alternative to perform the 2<sup>nd</sup> ANOVA, which improved the field trial error term by a 41.1% (44760/108889).

Factor	ANOVA	df	Mean square	P-value	Contribution
Ploidy	Without covariate	2	209407	0.151	6.24%
	With covariate	2	139781	0.047	1.97%
N-fertilization	Without covariate	2	72781	0.415	2.17%
	With covariate	2	77339	0.191	1.09%
Plant density	Without covariate	1	2962550	0.000	88.34%
	With covariate	1	5129	0.736	0.07%
Covariate	Without covariate	-	-	-	-
	With covariate	1	6842430	0.000	96.24%
Error	Without covariate	110	108889		
	With covariate	110	44760		

Kriging process estimates were calculated by the weighted sums of the adjacent topsoil  $k(h_0)$  values.

The results in the map (Figure 2) show areas with high  $k(h_0)$  values, which were associated with higher plant density, and with a 70-cm distance spatial resolution. Spatial errors determined by comparing experimentally measured and estimated  $k(h_0)$  values obtained from the spatial model are shown in Table 3. The effects of different factors on the  $k(h_0)$  values, by either considering kriging-estimated errors as a covariate (obtained by the geostatistical analysis) or not, are shown in Table 4. In the first case, only plant density proved statistical significance, with an 88.3% contribution factor. No differences due to either the species type factor or nitrogen fertilization factor were detected. However, when tKriging process residual values were considered as a covariate, differences were attributed to the species type factor because the covariate removed soil properties contribution due to the existing spatial variability in the experimental field. Even though plant cover density had no effect on unsaturated  $k(h_0)$ , *B. hybridum* showed a significantly higher unsaturated  $k(h_0)$  across topsoils than the other two species.

### Brachypodium biomass partition assessment

The four *B. hybridum* lines had bigger seeds than the *B. stacei* and *B. distachyon* lines. As a result, their seed germination was better and their crops were more viable in dry habitats. In fact, *B. hybridum* individuals were also the biggest plants, and were affected by certain genetic gigantism; i.e. their plants had a high leaf proportion on a dry weight basis, and they obtained higher root system/aerial part ratio values (Table 5) and a longer specific root length ( $\text{SRL} = 13.08 \pm 4.24 \text{ km}\cdot\text{kg}^{-1}$ ).

## Discussion

*Brachypodium* plant cover of croplands may play a relevant role in Mediterranean agricultural soils. For example, only 11.4% of Spanish agricultural soil can be used for optimum olive grove farming, and about a 44% cropland area is estimated to be affected by desertification [51], mainly because of soil erosion on slope land. Increasing soil water infiltrability and conductivity establishing a plant cover between the rows of woody crops, such as olive groves, vineyards, and other dry-fruit tree orchards [3], significantly reduces

**Table 5:** Number of plants sampled in the two tested seed density values, and plant biomass partition main parameters measured for *Brachypodium hybridum* (2n=30) plants.

	Mean	Range	SD
<b>Plant density / Number of samples*</b>			
LOW CC		6 - 9 (11 - 28)	$\pm 0.03$
REG CC		18 - 31 (19 - 46)	$\pm 0.03$
<b>Plant biomass partition</b>			
Volume	0.055	0.014 - 0.099	$\pm 0.027$
Weight	0.102	0.028 - 0.191	$\pm 0.052$
Specific root length (SRL), $\text{cm}/\text{mg}$	1.308	0.784 - 2.029	$\pm 0.424$
Leaf weight ratio (LWR), g/g	0.413	0.484 - 0.552	$\pm 0.026$
Steam weight ratio (SWR), g/g	0.300	0.286 - 0.319	$\pm 0.013$
Root weight ratio (RWR), g/g	0.287	0.153 - 0.228	$\pm 0.038$
Aerial weight ratio (AWR), g/g	0.713	0.771 - 0.872	$\pm 0.039$

\*Number of plants sampled in 400  $\text{cm}^2$  of soil surface for 2 years. Values in parentheses are the number of plants in year 2. SD = standard deviations.

surface runoff and soil erosion [52],

A relation has been found between other cover crops, such as *Sulla* and *Atriplex* shrubs, and water content in soils [53], but the effectiveness of plant cover to reduce soil erosion has not yet been fully achieved when used to protect soils of woody crops in Mediterranean environments because of the existing water competition against the main crop, e.g. olive trees [3] or vineyards [54]. Nevertheless, a genuine crop cover based on autochthonous mountain species has previously been assayed providing good soil erosion reduction in olive groves [55]. In this essay, a cover crop based on the three species of the *Brachypodium distachyon* complex was designed to examine the association of its species and polyploid occurrence (*B. hybridum*) with its adaptation to environmental aridity [56].

*B. distachyon* can display certain germination success depending on both soil water content retained in its micro-pores and the surface of soil particles at suction, as provided by the matric water potential as follows: 2% germinated seeds  $< -1.5 \text{ MPa} < \Psi_m < -0.05 \text{ MPa} < 50\%$  germinated seeds [57]. This property affects seed germination and the viability of the different *Brachypodium* species and ecotypes here in selected as cover crops. Non-dormant seeds were germinated at suitable temperature and matric potential [58], similar to BdTR4A and BdTR4B lines of *Brachypodium distachyon sensu lato* reported in Filiz et al. [59]. In fact, *B. distachyon* seeds maintain a high germination rate under wild conditions, and a high auto-seeding capacity for these BdTR4A and BdTR4B lines that belong to the *B. hybridum* species (2n=30 cytotype; Vogel et al. [60]). Temperature was identified as the major driver of their growth rate [61]. In addition, *Brachypodium pinnatum* and *Brachypodium sylvaticum* seeds are able to remain viable in the ground for 5 years [62].

The plant density of the cover crop provided by the tested *Brachypodium distachyon* complex species resulted statistically significant, with an 88.3% contribution to the unsaturated  $k(h_0)$  value of the covered sandy loam compacted soil (Table 4). These results confirmed those addressed by Hooke and Sandercok [63], and by Ruiz-Colmenero et al. [54]. Nevertheless, soil infiltration responds to a complex mechanism that involves organic matter content

change, aggregated stability [64], total porosity [65] and pore space connectivity [66], all of which result in increased available water capacity [67,68] and soil water infiltration [69].

The statistical significance shown for BD corresponded to the tested NT, LOW CC and REG CC treatments. BD generally addresses the effects of decreasing pore volume and the size of the pores through which water moves. Nevertheless, infiltrability and unsaturated  $k(h_0)$  increased correlatively with soil compaction, which could be attributed to the development of secondary permeability, linked to the surfaces generated by the appearance of mini-cracks and root channels, where adsorbed water moved according to differences in matric water potential. The increase in unsaturated  $k(h_0)$  after cropping *Brachypodium hybridum* plants for 2 years ( $2n=30$ ), compared with single inbred lines, was similar to that reported by Rahman [70] for a sandy soil of SE Burkina Faso. The unsaturated  $k(h_0)$  in the no-tillage bare soil treatment (NT) decreased and fell within the 0.679-2.906  $\text{cm h}^{-1}$  range, which is in accordance to a soil compaction value of  $\text{BD} = 1562 \pm 50 \text{ kg m}^{-3}$ . Similar data have been previously reported to reduce rooting [71,72].

Soil compaction decreases the volume of pores in quantity and size terms; both consequences lower infiltration rates and unsaturated hydraulic conductivity [73-75]. Although dry BD in NT plots was lower than BD for LOW CC and REG CC, unsaturated  $k(h_0)$  and cumulative infiltration were also lower in NT plots than for plant cover treatments. This apparent paradox could be attributed to the disaggregation effect of soil structure by rain erosivity and soil erosion ability under bare soil conditions after a dry summer, when the first storm episodes clog pores and soil is affected by sealing when it becomes wet, and by crusting when it is dry [2,4].

Macro-pores volume and soil strength both limit root elongation rates in soil. Soil compaction hinders root growth, thus it is a soil quality-related parameter [76]. BD for the three treatments (NT, LOW CC and REG CC) showed mechanical impedance for both root and shoot growth [77]. The localised soil compaction position determines root and, subsequently, shoot growth responses. The root system of *Brachypodium* spp. is similar to that of wheat [78], which is mainly cultivated under similar climate conditions to those of the herein considered experimental fields, and corresponds in plant density and soil sand content. Nitrogen fertilization was not statistically significant for the sets of plots located in the trial field because the *B. distachyon* complex species had no such avidity for the fertiliser, and its root system was able to colonize relatively poor soils without further aid [7,79]. Nevertheless, using *Brachypodium hybridum* as a cover crop helped reduce net leaching nitrate-N losses, as previously reported by Delgado and Bausch [80] in soils covered by malting barley and winter rye crops.

Hydraulic conductivity showed spatial variability. The second ANOVA was performed using the residual values obtained by Kriging to reduce this spatial soil disturbance, and to separate the species type and ploidy factors of *Brachypodium distachyon*. Significant differences in unsaturated  $k(h_0)$  linked to the species type and ploidy level of the plant lines were difficult to assess. Nevertheless, the individuals of *B. hybridum* ( $2n=30$ ) displayed better root system development (Table 5) compared with *B. distachyon* ( $2n=10$ ) and *B. stacei* ( $2n=20$ ) individuals; as well as a higher absolute average unsaturated  $k(h_0)$

value was also addressed.

Differences in biomass partition results could be attributed to soil compaction, as previously reported by Atwell [81] for wheat plants that grew in compacted soils in the U.K., where the RWR/SWR ratio was limited due to seminal root axes growth inhibition. Ecotypes of *Brachypodium hybridum*  $2n=30$  tend to be larger than ecotypes of *Brachypodium distachyon*  $2n=10$  [26]. Spanish *Brachypodium hybridum* grows faster and produces more biomass than polyploid lines [16,25]. The allotetraploid *Brachypodium hybridum* generally adapts better to drought conditions than the diploid *Brachypodium distachyon* due to its eco-physiological performance, including gas exchange, photosynthesis [2] and its “escapist” strategy to cope with aridity situations associated with water stress [56]. Plants with long roots and vast aerial green cover have also shown advantages for soil and water conservation, such as *Brachypodium hybridum* and the shrub *Atriplex halimus*, which have previously been addressed to increase soil macro-porosity [53,82]. Root growth is also proportional to the development of the aerial part [83]. Therefore, big plants develop big root systems, which improves soil water infiltration because the necessary preferential channels for water movement are precisely provided by root growth. This has already been shown for two commercial varieties of *B. distachyon* (Ibros and Zulema), which have been tested for interlines plant cover in olive groves in Spain [15]. *B. hybridum* also increased Soil Organic Matter content (SOM) near the root channel surface after 2 years of crop growing thanks to dead root decomposition and root channels abundance.

## Conclusion

In summary, *B. distachyon* ecotypes can protect soil and improve water soil infiltration. The cover of *Brachypodium distachyon*, *B. stacei* and *B. hybridum* plants positively affects soil properties and water conservation by reducing rain erosion effects. The aerial part of vegetation acts as a protective shelter against rainwater drop tapping, which would otherwise pull off and move soil fine particles of organic matter and clay. The hidden-half root part acts as a swelling and shrinking agent during wetting and drying processes, which increases soil-connected macro-pores where water moves. The use of residual values that derive from geostatistical analyses ensures relations between soil infiltration and plant cover density. Spatial analysis can be a new method for evaluating soil characteristics variability in plant breeding essays. A long developed Specific Root Length (SRL,  $\text{km kg}^{-1}$ ) is significant for increasing macro-porosity from death gross and medium roots, where water moves and is adsorbed on the surface of these root channels; whereas soil organic matter increases from fine roots decomposition. The adaptation of *B. hybridum* to Mediterranean environments characterizes this species as an interesting alternative to be used as a cover crop in woody crops, such as olive groves, vineyards or dry-fruit croplands.

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