

Isolated cork oak trees affect soil properties and biodiversity in a Mediterranean wooded grassland

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1 Isolated cork oak trees affect soil properties and biodiversity in aMediterranean wooded
2 grassland

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15

16 A B S T R A C T

17

18 Mediterranean wooded grasslands are multipurpose systems that support high plant and
19 animal diversity levels and are habitats of European importance (i.e., 6310 – Dehesas
20 with evergreen *Quercus* spp.). Moreover, these systems offer a number of agro-
21 ecosystem services such as forage production, soil carbon sequestration, nutrient
22 recycling and soil protection. The scattered trees enhance the ecological complexity of
23 grassland influencing the soil properties, the herbaceous layer diversity and
24 composition and the soil communities. Understanding how isolated trees influence the
25 other components of the system is essential to comprehend their role supporting high
26 levels of above and below ground biodiversity and ecosystem services.

27 In the present study, we present a hypothetical framework of the effects of isolated
28 trees on soil properties, plant and soil fauna assemblages, the latter here represented by
29 the class Collembola. The floor litter and the associated input of organic matter to the
30 soil was a key factor linking the components of the tree-soil-biodiversity system in a
31 Mediterranean cork oak wooded grassland.

32 Topsoil C increased by +50% under the tree canopy in comparison with the areas

33 beyond the tree canopy. Plant diversity was lower under tree canopy, but contributed
34 to enhance the total species richness of the grassland. Collembolan diversity was higher
35 under the peculiar conditions beneath the tree canopy. Relationships between plant and
36 collembolan species emerged.

37 The findings of this study suggest that isolated trees have direct and indirect effects on
38 soil properties, plant and collembolan assemblages, hence they can influence the
39 ecological processes of wooded grasslands, with implications for food webs, nutrient
40 cycling and productivity of the agro-ecosystem.

41

42

43 1. Introduction

44

45 In Mediterranean countries, agricultural landscapes are often dominated by scattered trees
46 with different tree densities, commonly referred as agro-silvo-pastoral systems (Moreno
47 and Pulido, 2012). These landscapes have evolved through millennia in the
48 Mediterranean Basin as a response to the environmental conditions and are associated with
49 a long history of deforestation, periodical fires and grazing as strategies to maximize
50 production of multiple goods and ecosystem services (Chirino et al., 2006; Pulido et al., 2010;
51 Zapata and Robledano, 2014). Similar complex landscapes can be found also in semi-arid
52 pastoral and savannah-type ecosystems worldwide, e.g., where woody encroachment was
53 and is still occurring (Asner et al., 2004; Tape et al., 2006; Liu et al., 2011).

54 Among the Mediterranean agro-silvo-pastoral systems, wooded grasslands are the most
55 widespread (Tárrega et al., 2009; Moreno et al., 2007; Eichhorn et al., 2006). In these
56 systems, cereal/fodder crops or semi-natural grass-herbs communities are mixed with a
57 tree layer mostly dominated by oak trees

58 (Pinto-Correia, 1993; Joffre et al., 1999; Carreiras et al., 2006; Costa et al., 2008). These
59 landscapes are common in Spain and Portugal, where they are respectively named Dehesa
60 and Montado, but are also spread in other areas such as Sardinia in Italy, where they cover
61 about 113,000 ha (4.7% of the regional surface and 9.8% of the total agricultural land) (RAS,
62 2013; ISPRA, 2014) (Fig. 2a). Sardinian wooded grasslands, often tilled and sown every two
63 to eight years to grow annual mixtures for grazing and/or hay production, are mainly
64 dominated by cork oak, with tree densities ranging from 7 to 250 ha⁻¹ and are generally

65 concentrated in the hilly areas of the North and the center. Grazing animals are principally
66 sheep and cattle (Eichhorn et al., 2006; Caballero et al., 2009; Rossetti and Bagella, 2014).

67 Mediterranean wooded grasslands are primarily productive systems, however their
68 relevance is not only associated to their economic importance: those with evergreen oaks
69 are also habitats of European importance (i.e., 6310 – Dehesas with evergreen *Quercus*
70 spp.) supporting high plant and animal diversity levels and serving as habitat for a number
71 of threatened species of the European fauna (Biondi et al., 2013). These multipurpose
72 systems offer a number of agro-ecosystem services such as forage production, soil carbon
73 sequestration, nutrient recycling and soil protection, and contribute to the esthetical value
74 of landscapes (Palma et al., 2007).

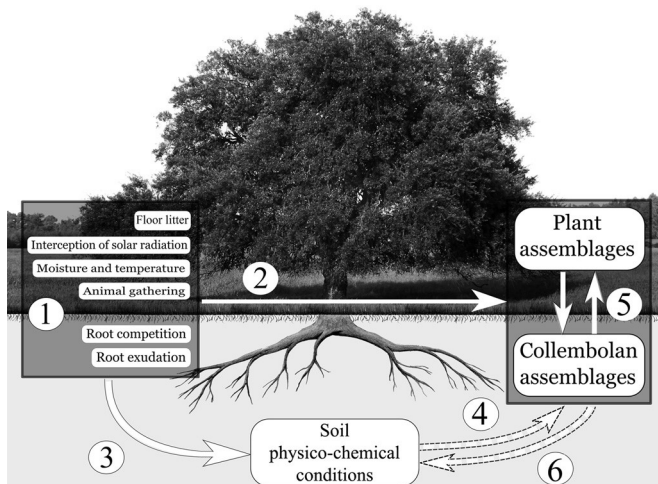
75 A key component of the most prominent features of wooded grassland is represented by
76 the scattered trees. The presence of trees in a farming system enhances its ecological
77 complexity.

78 The relationships between trees and understorey vegetation and soil properties have been
79 widely investigated in temperate and Mediterranean wooded pastures, forests and savannas
80 (Maltez-Mouro et al., 2005; Treydte et al., 2007; Perez-Ramos et al., 2008; Abdallah
81 and Chaieb, 2010; Canteiro et al., 2011; Abdallah et al., 2012; Xu et al., 2012). Interception
82 of solar radiation, root competition, litter humification and mineralization, cations and
83 water retrieval from deep soil layers may positively or negatively influence the herbaceous
84 communities under the tree canopy (Marañón et al., 2009). An increasing body of
85 knowledge is recently emerging on the role of trees in agroforestry systems on C
86 sequestration and on processes controlling C cycling in soils (e.g., Haile et al., 2008;
87 Takimoto et al., 2009; Nair et al., 2010; Pérez-Cruzado et al., 2012; Lai et al., 2014).
88 However, very few studies explored at small scale the effect of trees on the surrounding
89 biotic and abiotic environment (e.g., Fernández-Moya et al., 2010; Howlett et al., 2011),
90 and a small number are focused on the effects on soil fauna. For instance some groups of
91 pedofauna, like Collembola, are known as excellent bio-indicators of soil ecological
92 conditions. Not only they are an important element of the biodiversity in the soil system,
93 but they also respond to a variety of environmental and ecological factors, like changes in
94 soil chemistry, microhabitat configuration, and forestry and agricultural practices (Hopkin,
95 1997; Sousa et al., 2006). Sousa et al. (2004) found changes in species composition of
96 collembolan communities along a gradient of soil-use intensification in a Mediterranean

97 landscape dominated by cork oak. Nevertheless, to our knowledge, there is a lack of studies
98 on the impact of isolated oak trees on these bio-indicators for Mediterranean semi-arid
99 wooded grasslands.

100 In this study we examined the effect of isolated trees on soil properties, plant assemblages
101 and biotic soil components, the latter represented here by the Class Collembola.

102 We hypothesized that the effects of an isolated tree on soil properties, plant and
103 collembolan assemblages can be direct and indirect (see Fig. 1). We considered as direct
104 effects those derived from floor litter accumulation, interception of solar radiation, soil
105 moisture and temperature, root competition, root exudation and the animal gathering
106 (Haile et al., 2008; Marañón et al., 2009;



107
108
109 Fig. 1. Scheme of the hypothesized direct and indirect effects of the tree on soil
110 properties, plant and collembolan assemblages. Solid and dashed arrows represent,
111 respectively, direct and indirect effects of the tree on soil properties, plant and
112 collembolan communities. (1) Factors that exert a direct effect on soil properties,
113 plant and collembolan assemblages; (2) direct effects on plant and collembolan
114 assemblages; (3) direct effects on soil properties; (4) indirect effects of soil
115 properties on plant and collembolan assemblages; (5) reciprocal influence between
116 plant and collembolan assemblages; (6) influence of plant and collembolan
117 assemblages on soil properties.

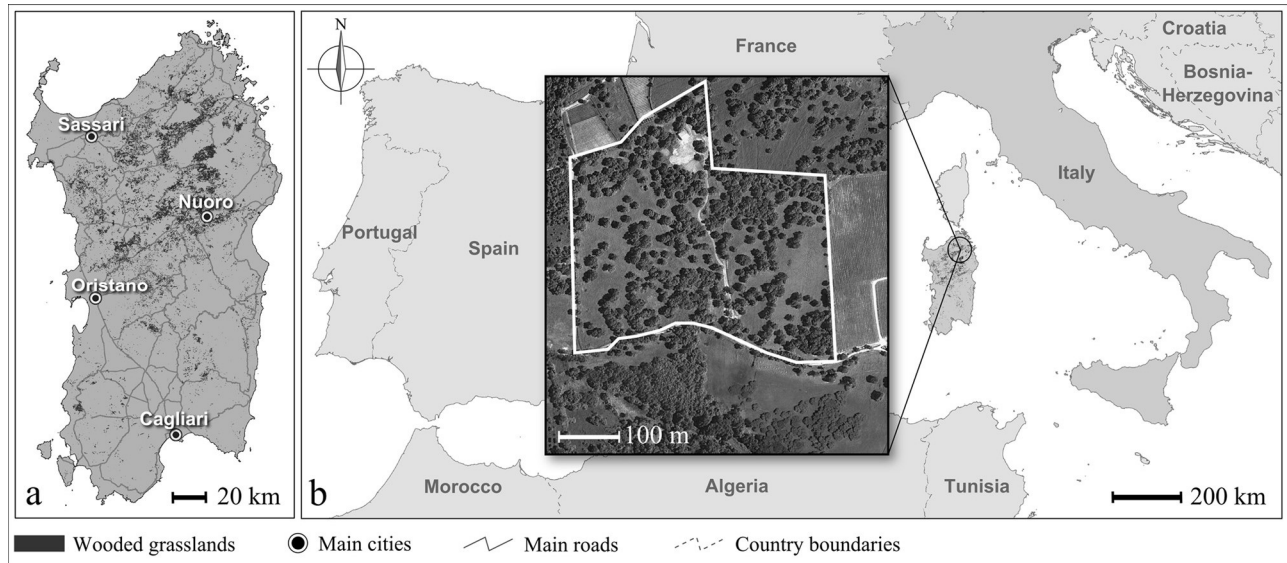
118
119 Takimoto et al., 2009; Nair et al., 2010; Pérez-Cruzado et al., 2012). We considered as
120 indirect effects of trees on plant and collembolan assemblages the soil properties that are

121 directly influenced by the trees via litter humification and mineralization. In turn, plant and
122 collembolan assemblages influence soil properties via organic matter
123 accumulation/decomposition, root exudation, nitrification and other complex mechanisms
124 involving soil food webs (De Deyn et al., 2003; Van der Heijden et al., 2008; Sabais et
125 al., 2012; Krab et al., 2013a; Hodson et al., 2014).

126 Because the role of interception of solar radiation, soil moisture and temperature on above
127 and below ground communities is well known (Moreno, 2008; Marañón et al., 2009;
128 Fernández-Moya et al., 2010), we focused our attention on floor litter as one of the most
129 prominent factors by which the tree exerts its direct and indirect effects on soil
130 properties and plant/collembolan assemblages. In our hypothesis we considered the floor
131 litter and the derived input of organic matter as the key factor linking the components of
132 the tree-soil-biodiversity system in Mediterranean wooded grasslands. The floor litter has
133 direct consequences on the input of organic matter on the soil and on the plant and
134 collembolan assemblages. In a medium and long term, the floor litter, affecting soil
135 properties, indirectly influences plant and collembolan assemblages through complex
136 interactions and feedback processes (Six et al., 2004; Krab et al., 2013a). Moreover, the
137 plant composition, by influencing litter quality, can have an impact on abundance, diversity
138 and diet choices of Collembola (Krab et al., 2013a). On the other hand, Collembola can
139 alter the soil organic matter decomposition patterns and, thus, plant nutrient acquisition and
140 performances that may modify plant competition and shape plant assemblages (De Deyn et
141 al., 2003; Sabais et al., 2012).

142 The objectives of this study were to assess in a Mediterranean
143 wooded grassland (i) the influence of isolated trees on soil properties, plant and
144 collembolan assemblage diversity and composition; (ii) the indirect effect of trees on plant
145 and collembolan assemblage composition via soil properties and (iii) the relationships
146 between plant and collembolan assemblage patterns.

147



148

149

150 Fig. 2. Distribution of wooded grasslands in Sardinia (a) and location of the study
 151 area (b).

152

153 2. Materials and methods

154

155 2.1. Study area

156

157 The study area (Fig. 2b) is located in the Long-Term Observatory of Berchidda-Monti,
 158 Sardinia, Italy (40° 47'0" N 09° 10'0" E, 320 a.s.l.). According to Rivas-Martínez and Rivas-
 159 Saenz (2014) the climate is Mediterranean pluvisessional oceanic, low mesomediterranean.
 160 The mean annual rainfall is 632 mm, 70% occurring from October to March; the annual
 161 reference evapotranspiration is 1193 mm, corresponding to an aridity index of 0.53. The
 162 mean annual temperature is 14.2 °C (range: 13.8–16.4). The prevailing wind direction is
 163 from South-West and the long-term average monthly wind speed ranges between 0.8 m s⁻¹
 164 and 1.4 m s⁻¹ with about 20% of gentle to moderate breeze according to the Beaufort
 165 scale. The soil developed from granitic rock, a parent material largely diffused in Sardinia
 166 (Carmignani et al., 2008), and was classified as Typic Dystrocherept (USDA, 2010). Soil
 167 texture in the Ap horizon is sandy loam (USDA, 2010) with average pH of 5.7 (range 5.1–
 168 6.4), organic C 2.3% (1.3–6.0) and total N 0.2% (0.9–5.4) (Seddaiu et al., 2013). The natural
 169 potential vegetation is mainly represented by *Quercus suber* L. forests referable to *Violo*

170 *dehnhardtii-Quercetum suberis* association (Bagella and Caria, 2011). The landscape is
171 character- ized by a wide variety of land uses practiced since centuries in the
172 area, related with different types of agro-silvo-pastoral activities which are dominated by
173 livestock farming (Le Lannou, 1979; Angius, 2006; Bagella et al., 2014a,b). Grazing
174 animals are mainly sheep and cattle and stocking rates range, respectively, from
175 0.7 to 2.2 LU ha⁻¹ and from 0.4 to 0.8 LU ha⁻¹ (1 LU = 500 kg cattle live weight = 6.6
176 dairy ewes). Cork oak wooded grasslands represent the dominant land use type covering
177 30% of the area. Woodlands are reduced to small patches with 50–90% tree cover and are
178 mainly exploited for cork production. Grape-growing represents a relevant source of
179 income (Bagella et al., 2013; Rossetti and Bagella, 2014).

180

181 2.2. *Experimental design*

182

183 Six isolated cork oak trees in a wooded grassland with a density of 30 trees ha⁻¹ on a
184 gentle slope (<5%) were randomly selected. The trees had a crown diameter of 11.8 SE
185 0.9 m and were 10.1 SE 0.3 m in height and 35.3 SE 1.2 cm in diameter at breast height.
186 The wooded grassland was managed since decades with
187 a flexible rotational scheme consisting of a fallow pasture periodically cropped, depending
188 on the dynamics of the thorny vegetation, with an annual hay crop mixture of annual
189 grasses (oats and Italian ryegrass) and annual clovers (Seddaiu et al., 2013). Grazing animals
190 were Sarda dairy sheep. Grazing occurred during

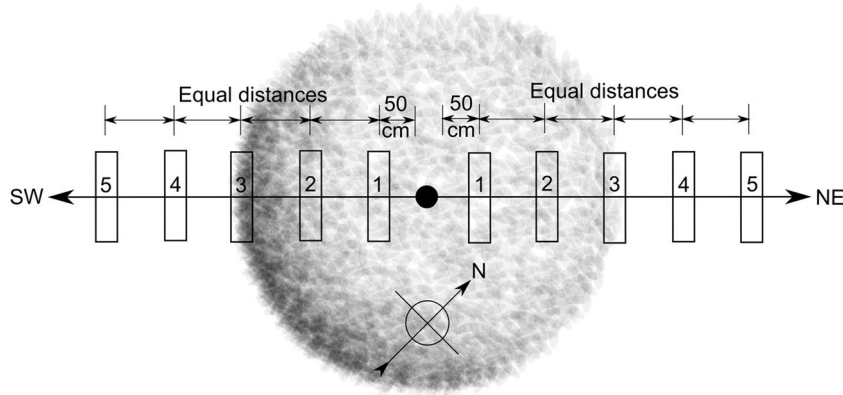
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192 the whole year with an average stocking rate of 3 ewes ha⁻¹ (Bagella et al., 2013).

193 The surveys were performed at each selected tree along two transects with opposite
194 orientations (North-East, NE and South-West, SW) following a scheme similar to that
195 adopted by Fernández-Moya et al. (2010). For each transect, surveys were made at
196 five regularly distanced sampling positions (Fig. 3), identified by taking into account
197 the horizontal projection of the canopy onto the ground: positions 1 and 2 were fully
198 underneath the tree canopy; position 3 was at the edge of the canopy; and two positions
199 were beyond the tree canopy (positions 4 and 5).

200 All the studied variables were measured at each sampling date in all the positions along
201 the two transects for a total of 60 sampling units (6 trees 2 transects 5 positions).

202 Collembola were collected from three positions, i.e., 1,3,5, in five of the six trees for a
203 total of 30 sampling units (5 trees 2 transects 3 positions).



204

205

206 Fig. 3. Sampling positions (1–5) along the two transects SW and NE. Full dot
207 represents the trunk.

208

209

210

212

211

213 2.2 Data collection

214

215 2.2.1. Floor litter

216 The floor litter was collected twice at each sampling unit in May and November 2011,
217 within a 25 × 25 cm² quadrat. The mineral soil was separated from the floor litter using a
218 brush and a spoon (Hoosbeek and Scarascia-Mugnozza, 2009). The sampling dates were
219 chosen in order to correspond to the two main peaks of litter fall for cork oak as reported
220 by Caritat et al. (2006) and Andivia et al. (2010). Litter samples were then oven-dried at
221 60 °C until constant weight.

222

223 2.2.2. Soil physico-chemical properties

224 In May 2011, at each sampling unit, three soil samples were collected with an
225 auger (5 cm diameter) at a 0–20 cm depth and mixed to form a unique sample. In a previous
226 study (Seddaiu et al., 2013) and under similar conditions to our study site (Howlett et al.,
227 2011) the 0–20 cm soil layer was found to be the most sensible one to differences of soil
228 management and distances to tree.

229 The pH was measured potentiometrically in the supernatant of a suspension with
230 1:2.5 soil:liquid ratio, using distilled water. Soil available P was determined by the Olsen
231 method (Olsen et al., 1954). The cation exchange capacity (CEC) and the exchangeable K⁺
232 were determined using BaCl₂–triethanolamine, buffered at pH 8 as a saturation solution
233 (MIPAF, 2000). A LECO CHN 628 elemental analyzer was used to determine C and N
234 contents.

235

236 2.2.3. Plant assemblages

237 Vegetation surveys were carried out in April and May 2011. A 2.0 × 0.5 m² plot divided
238 in 20 sub-plots was used for the surveys. All the plant species rooted inside each sub-
239 plot were recorded in a list. The frequency of each species at each sampling position was
240 assessed on the basis of the number of sub-plots in which it was found. For each species
241 the maximum frequency value found between the two sampling dates was considered for
242 data processing.

243 Species richness was calculated as the cumulative value of all identified species at each
244 sampling position over the two sampling dates. Shannon index (Shannon and Weaver,
245 1949) was also assessed. Plant nomenclature follows Pignatti (1982).

246

2472.2.4. *Collembolan assemblages*

248 Collembola were sampled in November 2011 taking a soil core (5 cm diameter) including
249 the organic horizon (when present) plus 10 cm in depth of the mineral soil. The soil cores
250 were taken for 5 trees in the positions 1, 3 and 5 of each transect, as species assemblages
251 on positions 2 (under the canopy) and 4 (outside the canopy) would be similar to the ones
252 observed in positions 1 and 5, respectively, and would not add much more information on
253 collembolan communities related to the three different habitat typologies (below the
254 canopy, interface area and outside the canopy). Collembola were extracted using dynamic
255 behavioral methods, based on an apparatus used to extract living micro-arthropods from
256 soil samples. In this method, a temperature gradient is created over the sample, such that
257 collembolan specimens tend to move away from the higher temperatures, toward the
258 bottom of the soil core, and fall into a collecting vessel filled with ethanol (70%), where
259 they are preserved for sorting and determination (Macfadyen, 1961). All collected
260 specimens were identified to the species or genus level.

261

262 2.3. *Statistical analyses*

263

264 A two-way analysis of variance (ANOVA) was carried out for floor litter, soil properties,
265 plant and collembolan assemblage richness and Shannon index, and collembolan
266 abundances (total individuals recorded within each soil core) according to a balanced
267 hierarchical design with sampling positions nested in the transects. Homogeneity of
268 variances was verified using the Cochran C-test, and data were appropriately transformed
269 when necessary (Underwood and Chapman, 1998). Mean comparisons were carried out
270 using the Student–Newman–Keuls test (Newman, 1939; Keuls, 1952). Pearson's
271 correlation coefficients were calculated among all soil variables and floor litter.

272 To evaluate which variables were effective in shaping plant and collembolan assemblage
273 composition two separated redundancy analyses (RDAs 1 and 2) were performed. Variables
274 with variation inflation factors >20 were not included in the analysis to prevent high
275 collinearity (McCune and Grace, 2002). The response variable matrices for RDAs 1 and 2
276 were composed by 60 sampling plots n plant species and by 30 sampling plots n
277 collembolan species respectively. Prior to run the RDAs, two detrended correspondence
278 analyses (DCA 1s and 2) were performed for plants and Collembola to delineate the basic
279 compositional gradients in the data and in order to select the appropriate constrained
280 ordination (Ter Braak and Šmilauer, 2002). The statistical significance of axes 1 and 2 was

281 tested by a Monte Carlo test with 499 permutations under the full model.

282 To detect possible relationships between plant and collembolan assemblage patterns the
283 co-variation of the composition of the two groups was evaluated using the Spearman's
284 correlation coefficient by comparing richness, Shannon index and the similarity matrices
285 based on Bray–Curtis similarity indices. Spearman's correlation coefficients between plant
286 species frequencies and Collembolan species abundances were calculated in order to find
287 out correlations between plant and collembolan species.

288 ANOVAs were performed with the SAS software (SAS Institute, 1999). RDAs were
289 performed with CANOCO v4.5 for Windows (Lepš and Šmilauer, 2003).

290

291 3.Results

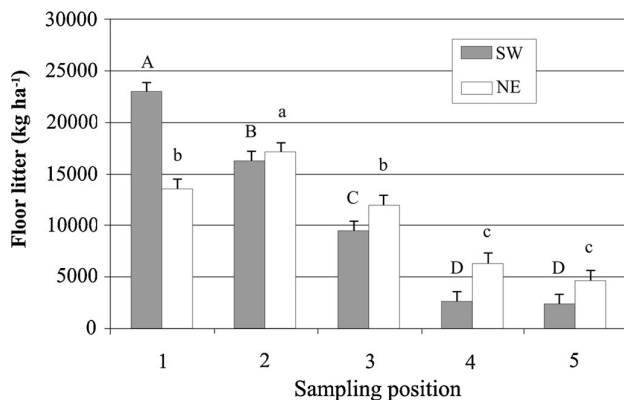
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293 3.1. Floor litter

294

295 The average amount of floor litter (10.7 t ha^{-1}) did not significantly differ between the
296 NE and SW transects, while significant differences were found among sampling positions
297 within transect.

298 In the SW transect, the floor litter dry matter decreased progressively from position 1 to
299 4, the latter position being similar
300 to 5 (Fig. 4). On average, the litter dry matter in the positions 4 and 5 was 88% lower than
301 the average amount in positions 1 and 2. In the NE transect, no significant differences were
302 found between the positions 1 and 3 that had significant lower floor litter biomass than the
303 position 2, while the litter in positions 4 and 5 was
304 significantly lower (on average 64%) than under the tree canopy projection.



305

306 Fig. 4. Floor litter dry matter (t ha^{-1}) in the North-East (NE) and South-West (SW)
307 transects. Mean values for each sampling position (see Fig. 3) within NE with
308 different lower-case letters significantly differ for $p \leq 0.05$. Mean values for each

309 sampling position within SW with different capital letters significantly differ for p
310 ≤ 0.05 . Bars represent the standard errors of the mean. –

311

312 *3.2. Soil properties*

313

314 The soil properties did not show significant differences between the two transects. The
315 sampling positions along the two transects had a significant effect apart from the C/N
316 ratio (Table 1). The pH in the closest position to the tree had the lowest values. The
317 available P and CEC showed significantly higher values beneath the tree canopy than
318 beyond the canopy projection with sharper differences in the SW transect. The
319 exchangeable- K^+ had a similar pattern, but with a lower value in the position 1 than in
320 the position 2. C and N values sharply decreased in the vicinity of the tree trunk between
321 the positions 1 and 2, while no significant differences among the position at the edge of
322 the canopy and the two outer positions were found.

323 Most of the variables were highly correlated each other, apart from the C/N ratio that
324 showed no significant correlation with any of the other variables (Table 2). Floor litter was
325 significantly correlated with all the soil properties, while available P and CEC were not
326 correlated with pH and C and N contents.

327

328 *3.3. Plant assemblages*

329

330 Overall, 131 plant species were identified; 13 of them are related to the EU priority habitat
331 type 6220*-Pseudo-steppe with grasses and annuals of the *Thero-Brachypodietea* (Biondi
332 et al., 2013; Bagella et al., 2014a) (Appendix A Table A1).

333

Table 1

Means and results of the analysis of variance for pH, available P (mg P₂O₅ kg⁻¹), exchangeable K⁺ (mg kg⁻¹), cation exchange capacity (CEC, meq kg⁻¹), contents of organic C (C, g kg⁻¹) and total N (N, g kg⁻¹), and C/N ratio in the top soil layer (0–20 cm) in relation to the transect orientation and sampling position.

Transect	Position	pH	Available P	Exchangeable K ⁺	CEC	C	N	C/N
North-East	1	5.5b	24.9ab	6.0bc	155a	36.9a	2.50a	14.7a
	2	5.8a	27.4a	9.0a	147a	29.7b	2.01b	14.9a
	3	5.9a	18.7b	7.3ab	137b	26.9bc	1.84bc	14.6a
	4	5.9a	18.6b	4.8bc	123c	24.5bc	1.60c	15.4a
	5	5.9a	18.7b	4.0c	123c	22.3c	1.57c	14.1a
	Mean	5.8A	21.7A	6.2 A	137A	28.0A	1.91A	14.7A
South-West	1	5.6b	23.7a	7.0ab	166a	42.8a	2.91a	15.0a
	2	5.8a	19.9ab	9.0a	150b	34.8b	2.40a	14.6a
	3	6.0a	16.2ac	7.3ab	139bc	29.0c	1.95b	14.9a
	4	5.9a	8.7c	5.0bc	126c	23.0c	1.56b	14.9a
	5	6.0a	12.5bc	4.6c	131c	23.5c	1.57b	14.9a
	Mean	5.8A	16.2A	6.6A	143A	30.6A	2.08A	14.9A
	CV(%)	2.7	42.3	29.5	6.8	12.2	15.0	6.8
Source of variation	d.f.	p values						
Transect	1	0.622	0.129	0.782	0.552	0.584	0.593	0.531
Position(transect)	8	<0.001	0.027	<0.001	<0.001	<0.001	<0.001	0.732

Mean values among sampling positions within transect with different lower-case letters significantly differ for $p \leq 0.05$. Mean values between transects with different capital letters significantly differ for $p \leq 0.05$.

334

Table 2

Pearson's correlation coefficients among litter dry matter yields and soil properties measured from ten positions with respect of the tree trunk.

	Floor litter biomass	pH	Available P	Exchangeable K ⁺	CEC	C	N	C/N
Floor litter biomass	1							
pH	-0.66*	1						
Available P	0.80**	-0.67*	1					
Exchangeable K ⁺	0.77**	-0.17	0.55	1				
CEC	0.91***	-0.77**	0.66*	0.66*	1			
C	0.90***	-0.82**	0.64*	0.57	0.97***	1		
N	0.89***	-0.82**	0.64*	0.56	0.97***	0.99***	1	
C/N	-0.12	0.22	-0.19	-0.16	-0.15	-0.12	-0.22	1

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$, ($n = 10$).

335

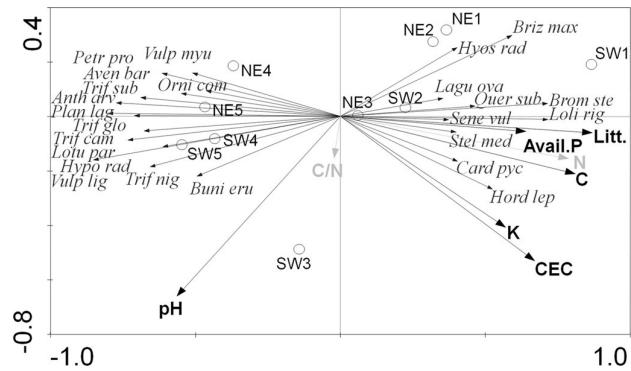
Table

3 Means and results of the analysis of variance for plant species richness and Shannon index in relation to the transect orientation and sampling position.

Transect	Position	Species richness	Shannon index
North-East	1	33.0a	3.1a
	2	29.5a	3.0a
	3	44.7b	3.4b
	4	46.2b	3.4b
	5	47.0b	3.4b
	Mean	40.1A	3.3A
South-West	1	31.8 a	3.1a
	2	38.2 a	3.3b
	3	46.8 b	3.4b
	4	46.2 b	3.4b
	5	48.0 b	3.5b
	Mean	42.2 A	3.3A
CV(%)	21.4	7.1	
Source of variation	d.f.	p values	
Transect	1	0.669	0.611
Position(transect)	8	<0.001	<0.001

Mean values among sampling positions within transect with different lower-case letters significantly differ for $p \leq 0.05$. Mean values between transects with different capital letters significantly differ for $p \leq 0.05$.

336



337

338 Fig. 5. Biplot from the RDA1 showing the position of (i) plant species and (ii)
 339 significant (plain black arrows) and not significant (plain grey arrows) variables. pH
 340 = pH in water, Avail.P = available P, Litt. = floor litter, K = exchangeable K^+ ,
 341 CEC = cation exchange capacity, C = contents of organic C, N = total N, C/N = C/N
 342 ratio in the top soil layer. Empty circles indicate the sampling positions. Only the 25
 343 most significant species are shown in the biplot.

344

345

346

347

Table 4

Means and results of the analysis of variance for collembolan species richness, Shannon index and abundance in relation to the transect orientation and sampling position.

Transect	Position	Species richness	Shannon index	Abundance
North-East	1	3.2a	0.8a	31.8a
	3	3.0a	0.8a	16.6ab
	5	0.8b	0.1a	2.2b
	Mean	2.3A	0.5A	16.9A
South-West	1	2.6b	0.6a	21.6a
	3	4.0a	1.0a	15.8ab
	5	2.3b	0.7a	5.8b
	Mean	3.0A	0.8A	15.0A
	CV(%)	40.9	45.1	109.3
Source of variation	df	p values		
Transect	1	0.205	0.181	0.744
Position(transect)	8	0.025	0.081	0.008

Mean values among sampling positions within transect with different lower-case letters significantly differ for $p \leq 0.05$. Mean values between transects with different capital letters significantly differ for $p \leq 0.05$.

348

349

350 Species richness and Shannon index did not show significant differences between the
351 two transects. Species richness was lower in the positions 1 and 2 than in the others
352 in both transects. A similar trend was observed for Shannon index (Table 3).

353 Sixteen species (12.2% of the total flora) were found exclusively in the positions close to
354 the trees (i.e., 1, 2 and 3) (Appendix A Table A1). The DCA1 ordination of plant
355 assemblages showed a strong gradient along the first axis (56.2% of the variance explained),
356 while along the second axis a clear gradient was not observed (6.7% of the variance
357 explained).

358 RDA1 (Fig. 5) shows the gradients associated to plant assemblages. Plant species
359 positioned on the right side of the x axis were more frequent under the tree canopy.
360 Plant species positioned on the left side of the x axis were more frequent beyond the canopy.
361 Among the eight explanatory variables included in RDA1, regarding the effects of the
362 environmental variables on plant assemblage composition, six, namely litter, available P,
363 pH, K, CEC, and C were significant (Table 5). Among them, floor litter explained the
364 highest percentage (57.1) of the total variance. Plant species associated with higher values
365 of litter, available P, K, CEC, C and lower values of pH were positioned on the right side

366 of the graph (Fig. 5). Conversely, plant species associated with lower values of litter,
367 available P, K, CEC, C and higher values of pH were positioned on the left side (Fig. 5).

368

369 3.4. Collembolan assemblages

370

371 Overall, 12 collembolan *taxa* were identified (Appendix A Table A2).

372 Collembolan abundance mean values, species richness and Shannon index did not show
373 significant differences between the two transects. In both transects abundance of
374 collembolan individuals was significantly higher in the positions 1 and 3 (under the canopy
375 and edge, respectively), than in the position 5 (i.e., beyond the tree canopy). Species
376 richness was also significantly higher in the positions 1 and 3 of the NE transect and in
377 the position 3 of the SW transect than in the other positions. No differences were observed
378 for Shannon index (Table 4). Seven *taxa* (58.3% of the total) were found exclusively in
379 the positions close to the trees (positions 1 and 3) (Appendix A Table A2).

380 The first axis of the DCA2 ordination of collembolan assemblages explained the 57.4%
381 of the variance, while along the second axis there was no evidence of a clear gradient
382 (2.2% of the variance explained).

383 RDA2 (Fig. 6) shows the gradients associated to collembolan assemblages. *Isotoma* sp. and
384 *Cryptopygus* c.f. *albaredai* were more correlated with the position SW3. *Folsomia*
385 *quadrioculata* was more correlated with the position NE1. Among the eight explanatory
386 variables included in RDA2, regarding the effects of the considered variables on
387 collembolan assemblage composition, only pH was significant (Table 6). Collembola
388 associated with lower pH were positioned on the right side of the *x* axis (Fig. 6).

389

390

Table 5
Explanatory variables and related explained variance resulting from RDA1.

Variable	Lambda A	p value	F	% of explained variance
Floor litter biomass	0.23	0.002	17.25	57.1
Available P	0.05	0.002	4.06	12.4
pH	0.03	0.002	2.57	7.4
Exchangeable K ⁺	0.03	0.002	2.20	7.4
CEC	0.02	0.014	1.99	5.0
C	0.02	0.050	1.56	5.0
N	0.01	0.406	1.01	2.5
C/N	0.01	0.544	0.93	2.5

391

392

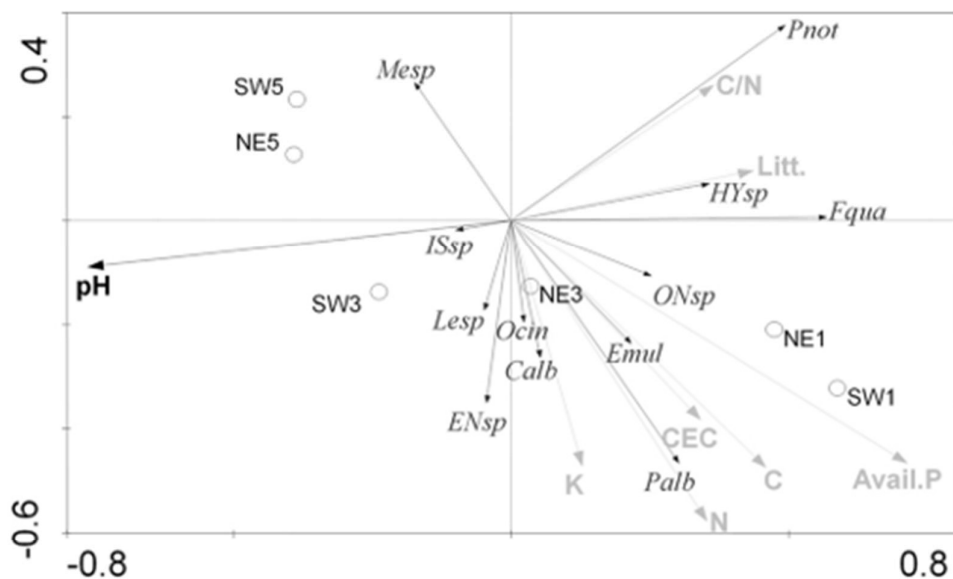


Fig. 6. Biplot from the RDA2 showing the position of (i) collembolan species and (ii) significant (plain black arrows) and not significant (plain grey arrows) variables. pH = pH in water, Avail.P = available P, Litt. = floor litter, K = exchangeable K⁺, CEC = cation exchange capacity, C = contents of organic C, N = total N, C/N = C/N ratio in the top soil layer. Empty circles indicate the sampling positions.

393

Table 6
Explanatory variables and related explained variance resulting from RDA2.

Variable	Lambda A	p value	F	% of explained variance
pH	0.16	0.010	4.96	47.5
C/N	0.04	0.180	1.43	11.9
Available P	0.05	0.162	1.83	14.8
N	0.03	0.440	0.72	8.9
C	0.03	0.260	1.03	8.9
Floor litter biomass	0.01	0.644	0.42	3.0
Exchangeable K ⁺	0.01	0.824	0.26	3.0
CEC	0.01	0.788	0.26	3.0

394

395

396 3.5. Relationships between plant and collembolan assemblages

397

398 There was no significant relationship between diversity indices of plant and collembolan
 399 assemblages. The Spearman's correlation coefficient for species richness and Shannon
 400 index were respectively 0.134 ($p = 0.489$) and 0.049 ($p = 0.800$). The coefficient of correlation
 401 between Bray–Curtis similarity indices of plant and collembolan data was 0.118 ($p < 0.017$).

402 Among the collembolan taxa, *Folsomia quadrioculata*, *Isotoma*

403 sp., *Pseudosinella alba* and, secondarily, *Entomobrya multifasciata*, manifested the most
 404 appreciable correlation with plant species (Appendix A Table A3). *F. quadrioculata*, that

405 was dominant in the positions under the tree canopy, showed a positive correlation
 406 especially with some grasses that were abundant under the canopy, such as *Briza maxima*,

407 *Bromus sterilis*, *Hordeum leporinum* and *Lolium rigidum*, and a negative correlation with

408 legume species of the genus *Trifolium* and other plant species that were dominant beyond
 409 the canopy. *P. alba* showed a similar pattern, but less evident than *F. quadrioculata*.

410 *Isotoma* sp., that was dominant in the edge of the canopy, showed the opposite trend and it
 411 was positively correlated with the *Trifolium* genus and negatively correlated with the grass

412 species that were dominant under canopy. In contrast to *F. quadrioculata* and *P. alba*, *E.*

413 *multifasciata* was also dominant under the canopy but showed stronger positive correlations

414 with *L. rigidum* and *Senecio vulgaris* and negative correlations with *T. subterraneum*,

415 *Vulpia ligustica* and *V. myuros*, that were dominant beyond the canopy. *Parisotoma*

416 *notabilis*, that showed a preference for the positions under the tree canopy, but was also

417 frequent in the edge and beyond the canopy, showed only a negative correlation with *T.*

418 *subterraneum*. *Mesaphorura* sp. was the only collembolan taxa that did not show a clear

419 gradient nor correlation with plant species.

420

421 4. Discussion

422

423 4.1. Floor litter

424

425 The floor litter was significantly higher under the tree canopy than in the open grassland,
426 while the amount of floor litter at the canopy edge was somehow intermediate between
427 under and beyond the tree canopy. The differences between positions within each transect
428 can also be partly attributed to local factors such as the direction of dominant winds that
429 also shapes the tree canopy symmetry and hence the litter deposition pattern. Moreover,
430 the prevailing wind in the study area is from South-West and this could help explaining the
431 relative high litter amount in the closest position to the tree in the South-West transect with
432 respect to the North-East one. In fact, we could hypothesize that the trunk could represent
433 a sort of barrier that facilitates a higher accumulation of the litter in the SW than in the
434 NE direction.

435 Under semi-arid Mediterranean conditions, Vacca (2000)

436 compared the forest floor litter features between a dense (400 trees ha⁻¹) *Q. suber*
437 forest mainly used for cork production and a wooded grazed grassland with about 40 *Q.*
438 *suber* trees ha⁻¹. The organic C content in the floor litter ranged from 10 to 22 t ha⁻¹ in the
439 dense oak forest and from 3 to 11 t ha⁻¹ in the wooded pasture. Assuming a litter C content
440 of 490 g kg⁻¹ of dry biomass from the oak trees (Vesterdal et al., 2012), our results were
441 comparable with those reported for the wooded pasture. On the contrary, for the positions
442 below the tree canopy projection our findings (on average, 8.5 t C ha⁻¹ in the floor litter)
443 were higher than those found by Hoosbeek and Scarascia-Mugnozza (2009), who never
444 observed values above 6.0 t C ha⁻¹ in a poplar plantation under Mediterranean conditions.

445

446 4.2. Soil properties

447

448 In the positions beyond the tree canopy the soil fertility level was medium, even if some
449 nutrients indicators (i.e., available P, CEC) were only slightly above the upper limit for
450 the low fertility status reported for Italian soils (Landi, 1999). Only the range of the
451 exchangeable K⁺ was always above the maximum threshold (3.5 meq kg⁻¹) considered
452 for K-rich soils. The C content along the transects was two to four times higher than 1.0%,
453 which is the lower limit for identifying the status of pre-desertification (Commission of the

454 European Communities, 2002). In comparison to similar management and climate
455 conditions in Spanish dehesas, we found higher values of total N and exchangeable K^+ both
456 in the open areas and beneath the tree crown (Moreno and Obrador, 2007; Moreno et al.,
457 2007; Fernández-Moya et al., 2010). However, the C content values were consistent to what
458 found by Moreno and Obrador (2007) and Pulido-Fernández et al. (2013) for similar
459 acidic soils in holm oak wooded grasslands of Central and South-Western Spain.

460 Our study confirmed the general pattern of exponential decrease of most nutrients with
461 the distance from the tree, which was already reported by several authors under semi-arid
462 Mediterranean climate for the top soil (Moreno and Obrador, 2007; Moreno et al., 2007;
463 Howlett et al., 2011). A positive effect of trees on soil fertility has been reported also for
464 other climate conditions such as subtropical areas in Florida, USA (Haile et al., 2010),
465 semiarid-to-arid West African Sahel (Takimoto et al., 2009), tropical areas of Costa Rican
466 Atlantic coast (Amézquita et al., 2005).

467 The impact of silvo-pastoral management practices on soil C content depends largely on
468 the amount of biomass input provided by trees and other components of the system, such
469 as herbaceous vegetation and animal dejections, and on soil properties (Nair et al.,
470 2010 and references therein). In our study, floor litter, mainly associated to tree litterfall,
471 proved to be a suitable indicator of the C input to the soil as evidenced by the highly
472 significant correlation between this variable and soil C content. Similarly, Simón et al.
473 (2013) reported that soil C distribution is highly influenced by litterfall and that soil
474 characteristics are linked to the crown size. In particular, they highlighted a positive
475 correlation between tree presence and organic C stocks up to distances of 8 m from the tree
476 in a holm oak dehesa in Central Spain. Consistently, our results indicate that already around
477 6.0 m from the tree trunk (position 3) some soil fertility indicators, namely C and N
478 contents and CEC, were not significantly influenced by the presence of the tree.

479 According to Gallardo (2003) the similar spatial distribution of
480 C and N along the transects indicates that the soil total N content is basically controlled by
481 the soil organic matter mineralization processes. Similarly, lower pH values under vs.
482 beyond the tree canopy and the strong negative association ($r = 0.83$) between pH and C
483 content suggest that the pH pattern is mainly controlled by SOM.

484

485 4.3. *Plant assemblages*

486

487 Both species richness and Shannon index significantly decreased along the transects from

488 the open to the under canopy positions, as also observed by Fernández-Moya et al. (2010),
489 confirming the positive correlation between available photo-synthetically active radiation
490 (PAR) and plant diversity, and the negative correlation between soil fertility and plant
491 diversity. Interference factors, such as physical or allelopathic influence of floor litter
492 and soil compaction and N enrichment from animals gathering under the canopy to
493 exploit the tree shadow can also explain the lower species diversity under the tree canopy
494 (Marañón, 1986; Vilà and Sardans, 1999; Borchsenius et al., 2004; Casado et al., 2004;
495 Marañón et al., 2009; Rodríguez-Calcerrada et al., 2011). In the open areas, where the
496 PAR is higher but the soil fertility is lower, the competition for nutrients becomes the
497 dominant constraint, favoring higher levels of plant diversity because most plant species in
498 grasslands are adapted to moderate or low nutrient conditions (Klimek et al., 2007;
499 Fernández-Moya et al., 2010). However, a significant number of species were found
500 exclusively or mainly close to the trees, suggesting that the presence of isolated oak trees
501 in wooded grasslands considerably contributes to the overall plant diversity of the system.
502 The significant effects of litter on plant assemblages was
503 consistent to what observed by Rodríguez-Calcerrada et al. (2011) who reported that soil
504 litter accumulation is one way through which overstorey composition shapes the plant
505 communities under the tree canopy, because of its direct influence on the penetration of
506 PAR to the ground and on soil temperature and water content (Marañón et al., 2009) that,
507 in turn, have effects on seed germination and seedling establishment. Litter decom-
508 position is also responsible of nutrient release and, thus, on plant growth conditions
509 (Prevost-Boure et al., 2010; Vesterdal et al., 2012; Shvaleva et al., 2014).
510 Available P, pH, K, CEC and C also significantly contributed to explain the variation of
511 plant assemblages. Total N was highly correlated with litter and certainly it contributed in
512 shaping plant assemblages, even if it did not explain any significant additional variation in
513 the RDA1. Some species dominant under the tree canopy, i.e., *Briza maxima*, *Bromus*
514 *sterilis*, *Carduus pycnocephalus*, *Hordeum leporinum*, *Lolium rigidum* are known as
515 nitrophilous species, and are associated with higher fertility levels of the under canopy
516 environment.

517 Available P is recognized as one of the most important limiting factors affecting both
518 species richness and composition of grasslands. However, its effect can be profoundly
519 influenced because of the interaction with other factors such as pH, concentration of anions
520 and cations and also vegetation (Devau et al., 2009; Merunková and Chytrý, 2012).
521 Although annual legumes are recognized to be favored by soil P availability (De Ruiter,

522 1981; Olea and San Miguel-Ayanz, 2006), heliophilous species such as *Trifolium*
523 *subterraneum*, *T. nigrescens*, *T. campestre*, *Ornithopus compressus*, were mainly dominant
524 in the positions beyond the tree canopy, characterized by lower soil available P contents,
525 in accordance to what reported by Fernández-Moya et al. (2010).

526 The effect of soil pH on plant richness and composition in
527 grasslands is well known. At pH values lower than 6, the relationship between pH and
528 species richness is almost always positive (Ewald, 2003; Crawley et al., 2005; Olsson et
529 al., 2009; Merunková and Chytrý, 2012). Soil acidity influences the solubility of toxic
530 metals and mineral nutrients (Olsson et al., 2009; Balkovič et al., 2014). Soil P and N
531 availability are both strongly pH dependent. In acidic soils, P tends to form stable minerals
532 with Al and Fe, while ammonium (NH_4^+) and nitrate (NO_3^-) tend to increase and decrease
533 respectively, favoring those plant species able to take up ammonium (Bartelheimer and
534 Poschlod, 2014). Moreover, because acidic soils and high organic C concentration favor
535 decomposers and fungal communities, decomposition and depolymerization processes
536 increase the N and P availability (Delgado-Baquerizo et al., 2009). Soil pH plays a key
537 role also in legume nodulation and nitrogen fixation. The inability of several rhizobia
538 species to survive and form rhizobium–legume symbiosis in acidic soils is one cause of
539 nodulation failure (Zahran, 1999).

540

541 4.4. *Collembolan assemblages*

542

543 More than half of collembolan *taxa* were found exclusively in the positions 1 and 3,
544 implying a strong contribution of the trees on the total collembolan diversity in the studied
545 wooded grassland (Appendix A Table A2). Under the tree canopy the shaded conditions
546 promote moister and colder habitats which are the preferred conditions for most
547 Collembola, especially some eu-edaphic species (Hopkin, 1997; Krab et al., 2010).
548 Moreover, microhabitats under the canopy uphold a thicker and richer litter layer,
549 increasing the amount of organic matter available to soil fauna and that have been shown
550 to be beneficial to eu-edaphic microarthropods (Parisi et al., 2005), including most soil
551 dwelling collembolan species (Berg and Bengtsson, 2007).

552 Trees had a significant effect on collembolan species richness,
553 but not on Shannon index, due to the higher predominance of few species, particularly
554 *Folsomia quadrioculata*, within the positions under the tree canopy. Several hemi-edaphic
555 species such as *F. quadrioculata* have been showed to be moderately sensitive to drought

556 (Hertzberg and Leinaas, 1998) and to respond negatively to the temperature increase in the
557 topsoil layers (Krab et al., 2013b), so they were expected to be more associated to shaded
558 areas. Consequently, the higher dominance of species adapted to the habitat conditions
559 supported by the trees has led to a low evenness of the Collembolan communities beneath
560 the tree canopy. Differences in diversity values were thus not significant along the
561 transects.

562 On the other hand, part of epi-epidaphic and hemi-edaphic Collembola, e.g., some species
563 of the genus *Isotoma* and *Cryptopygus*, are more resistant to temperature and moisture
564 fluctuations (Hopkin, 1997; Kuznetsova, 2003; Sousa et al., 2004; Elnitsky et al., 2008)
565 and can be adapted to dryer habitat conditions and more grassy soil surfaces (e.g.,
566 Kuznetsova, 2002; Ponge et al., 2006). Therefore, species richness was higher at the
567 ecotone of the tree canopy, where the overlapping of habitat conditions of shaded and open
568 areas, has promoted a higher frequency of both xerophilous and hygrophilous collembolan
569 species.

570 Among all environmental variables, only pH seemed to have a significant effect on
571 Collembola. It is known that pH can exert some influence on collembolan physiological
572 functions (e.g., Van Straalen and Verhoef, 1997; De Boer et al., 2010) and community
573 structure (Rusek and Marshall, 2000). However, pH effects on collembolan communities
574 can be confounded with other abiotic factors influencing differently a particular species
575 (De Boer et al., 2010). The pH values recorded in this study were highly negatively
576 correlated with soil C and N contents along the transects. Thus pH could have reflected the
577 influence of SOM availability on certain collembolan species, and hence the indirect effect
578 of the under canopy environment by providing higher SOM levels in the thicker litter
579 layers. Hence, the independent effect of pH on collembolan community structure seems
580 difficult to grasp and caution is needed in the interpretation of these results.

581

582 4.5. *Relationships between plant and collembolan assemblages*

583

584 Although plant and collembolan species richness, Shannon index and assemblages were
585 not correlated, some collembolan species showed a significant correlation with many of
586 those plant species that manifested the most appreciable changes along the transects. *F.*
587 *quadrioculata* and *Isotoma* sp. were the Collembola most correlated with plants. *F.*
588 *quadrioculata* seemed mainly associated to grasses that were dominant under the canopy,
589 *Isotoma* sp. seemed to have a preference for legumes, such as

590 *T. subterraneum* and *T. nigrescens*, that were dominant beyond the canopy, instead. It is
591 difficult to explain these relationships, but it is possible to suppose a reciprocal influence
592 due to the effect of plants and Collembola on the litter quality and the nutrient availability
593 (Sabais et al., 2012). Moreover, the results showed that pH is the only soil variable that
594 significantly affected both plant and collembolan assemblages. Soil pH influenced plant
595 and collembolan species that were positively or negatively correlated each other. For
596 instance, *B. sterilis* and *F. quadrioculata* and *T. nigrescens* and *Isotoma* sp. These findings
597 suggest that pH could have a role influencing both plant and collembolan assemblages, or
598 it can be considered somehow as a proxy parameter reflecting the tree canopy effect. Soil
599 litter was negatively correlated with pH (Fig. 6 and Table 2), therefore it is possible to
600 assume also a direct role of litter on collembolan assemblages.

601

602 5. Conclusions

603

604 This study contributes to enhance the knowledge on the complex mechanisms involved
605 in the tree-soil-biodiversity system in Mediterranean wooded grasslands.

606 Soil C under the tree canopy was 50% higher than beyond the tree canopy and this was
607 directly associated to the variation of the floor litter biomass. The direct effect of trees on
608 soil properties, in turn, indirectly influenced plant and collembolan assemblages. In terms
609 of effects on biodiversity, while collembolan diversity seemed to be enhanced by the
610 peculiar conditions under the tree canopy, the plant species richness decreased under the
611 tree canopy. Many plant and collembolan species were associated to the conditions created
612 by the isolated trees, therefore the isolated trees contributed to enhance the total
613 biodiversity and ecological complexity of the entire system. Some plant and collembolan
614 species manifested negative or positive correlations, suggesting that various plant or
615 collembolan species are affected by the same environmental factors. We hypothesize that
616 some plant and collembolan species are also functionally connected, and further studies are
617 desirable to better explain the plant–Collembola relationships that emerged in this study.
618 Among the analyzed soil properties, pH seems to be the main variable that affects both
619 plant and collembolan assemblages, suggesting a role of this variable in shaping the
620 assemblages of these taxonomic groups, probably because pH summarizes the effect of
621 trees on both plants and Collembola.

622 It is not clearly proved by this study, but the higher amount of

623 organic C and abundance of Collembola under canopy, and the plant–Collembola

624 relationships emerged suggest that the isolated trees can have a role enhancing the soil food
625 webs in wooded grasslands, supporting the nutrient recycling and, in turn, improving the
626 fertility of the whole agro-ecosystem.

627

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629

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636

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638

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