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1 **Weed seedbank size and composition in a long-term tillage and crop sequence experiment**

2

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17

18 **Abstract**

19 Knowledge of the effects of agricultural practices on weed seedbank dynamics is essential for
20 predicting, and consequently limiting, future problems in weed management. This paper reports
21 data relative to weed seedbank structure after 18 years of continuous application of conventional
22 tillage (based on moldboard plowing) or no tillage within three crop sequences (continuous wheat;
23 wheat–faba bean; and wheat–berseem clover seed crop) in a typical Mediterranean environment.
24 The seedbank was monitored for two consecutive growing seasons using the seedling emergence
25 method. Compared to crop rotations (wheat–faba bean and wheat–berseem), the continuous
26 monoculture of wheat resulted in a great increase in total weed seedbank density and a reduction in
27 weed diversity, with a strong increase in some species, some of which (i.e., *Polygonum aviculare*
28 and *Lolium* spp.) are potentially hard to control. However, tillage system did not affect the size of
29 the weed seedbank but altered both its composition and the distribution of seeds along the soil
30 profile. In particular, the adoption of conventional tillage favored some species (mainly *P.*
31 *aviculare*), whereas the continuous use of no tillage led to an increase in weed seeds in the upper
32 soil layer and resulted in a significant increase in the seed density of some problematic species, such
33 as *Papaver rhoeas*, *Phalaris* spp., and *Lactuca serriola*. The effects of tillage system on weed
34 seedbank size and composition were less pronounced in the wheat–berseem clover crop rotation
35 than in either the wheat–faba bean or continuous wheat cropping systems.

36

37

38 **Introduction**

39 Although important advances in weed control technologies have been made in the past decades,
40 weeds are still a major concern in agricultural systems, able to cause significant losses in crop yield
41 and quality. Weeds remain one of the most detrimental factors in crop performance because weed
42 flora continually change in response to new control measures [1]. Several studies have documented
43 how weed flora respond to changes in agricultural practices [2,3]. Crop sequence and tillage system
44 are two primary practices that affect weed population dynamics [4,5]. Crop rotation has
45 traditionally been the simplest and most effective method of weed control [6], and many studies
46 have documented changes in both the weed seedbank community and the aboveground weed
47 community due to the adoption of different crop rotations [7,8]. Crop rotation increases weed
48 diversity and reduces weed density compared to monocultures [6], mainly because the selective
49 pressure on weed flora exerted in monoculture systems can over time favor the buildup of species
50 with a phenotype and phenology similar to that of the crop [9]. Obviously, the choice of crops and
51 the sequence in which crops are grown can markedly influence weed community dynamics because
52 of their different biological cycles, end use, competitive ability against weeds, cultural management
53 practices (fertilization, seedbed preparation, etc.), and, above all, weed control measures (the use of
54 herbicides, mechanical operations, etc.). Moreover, the response of the weed community to a
55 particular crop sequence can vary by environment; thus, it is not surprising that experiments
56 performed in environments with different climate and soil characteristics have found variable and in
57 some cases contradictory results.

58 Tillage can consistently affect the weed community, causing a vertical redistribution of seeds
59 along the soil profile and changes in soil characteristics (which in turn determine changes in soil
60 habitability and, as a consequence, advantage or disadvantage different weed species) and dictating
61 weed control management strategies (e.g., pre-emergence use of nonselective, broad-spectrum,
62 systemic herbicides under no tillage [NT]). Hence, it is not surprising that several studies have
63 shown strong variations in the size and composition of the weed seedbank in response to changing

64 tillage systems [1,5,10]. In general, both the abundance and diversity of soil communities increase
65 with decreasing tillage. In particular, several authors have observed an increase in annual grasses,
66 perennial weeds, and wind-dispersed species under NT [11,12]. Such floral changes under reduced
67 tillage (RT) or NT have been interpreted by some authors as steps in an ecological succession
68 [13,14]. However, other research has shown no alteration of the weed community in response to the
69 application of conservative tillage techniques [15,16].

70 Furthermore, tillage and crop rotation often interact to determine the composition and abundance
71 of weed species in crop fields [17], but contradictory results can be found in the literature. Doucet
72 & Hamill [18] reported that weed density was affected more by the tillage system than the cropping
73 system. Along these same lines, Bàrberi & Lo Cascio [10] concluded that the tillage system
74 influenced weed seedbank structure to a much greater extent than did crop rotation, and Brainard et
75 al. [19] stated that the impact of a particular crop sequence is often less important than the
76 management practices used in that sequence. However, Ball [4] reported that cropping sequence
77 was the most dominant factor influencing species composition in the weed seedbank.

78 Although studies on weed seedbank do not provide information on real weed density (because
79 only a small percentage of seeds present in the soil germinate each year), they are fundamental to
80 understanding and predicting the evolution of weed communities, as the seedbank reflects the
81 history of the field. Indeed, the size and composition of the weed seedbank is a consequence of the
82 cultural management practices applied, the crops grown, and the effects of these factors on weed
83 species and their fitness.

84 A long-term experiment was begun in 1991 to study the effects of the continuous use of
85 conservation tillage techniques (RT and NT) on the performance of crops in cereal–legume rotation
86 systems typical of the semiarid Mediterranean environment. The present paper reports data relative
87 to weed seedbank structure after 18 years of continuous application of the treatments. In particular,
88 we compared the size, composition, and diversity of the weed seedbank between conventional

89 tillage (CT, based on moldboard plowing) and NT within three crop sequences: continuous wheat
90 (W_W), wheat–faba bean (W_F), and wheat–berseem clover (W_B) seed crop.

91

92 **Materials and methods**

93 *Ethics Statement*

94 No specific permits were required for the described field study. The location is not protected in
95 any way. The experiment did not involve endangered or protected species.

96

97 *Site characteristics, experimental design, and management*

98 A long-term field experiment was started in the 1991–1992 growing season at Pietranera farm,
99 which is located about 30 km north of Agrigento, Sicily, Italy (37°30' N, 13°31' E; 178 m a.s.l.), on
100 a deep, well-structured soil classified as Chromic Haploxerert (Vertisol). Soil characteristics
101 (measured at the beginning of the experiment and referring to the 0- to 0.40-m layer) were as
102 follows: 52.5% clay, 21.6% silt, 25.9% sand, pH 8.1 (1:2.5 H₂O), 1.40% total C (Walkley Black),
103 1.29 g kg⁻¹ total N (Kjeldahl), 36 mg kg⁻¹ available P (Olsen), 340 mg kg⁻¹ K₂O (exchangeable
104 potassium), cation exchange capacity 35 cmol₊ kg⁻¹, 0.38 cm³ cm⁻³ water content at field capacity
105 (pF 2.5), and 0.16 cm³ cm⁻³ permanent wilting point (pF 4.5). The climate of the experimental site
106 is semiarid Mediterranean, with a mean annual rainfall of 552 mm, mostly in autumn/winter (74%)
107 and in spring (18%). The dry period is from May to September. The mean air temperature is 15.9°C
108 in autumn, 9.8°C in winter, and 16.5°C in spring. The average minimum and maximum annual
109 temperatures are 10.0°C and 23.3°C, respectively. Weather data were collected from a weather
110 station located within 500 m of the experimental site.

111 The experiment was set up as a strip-plot design with two replications. Three soil tillage systems
112 (CT, RT, and NT) acted as vertical treatments and three crop sequences (W_W , W_F , W_B) as
113 horizontal treatments. CT consisted of one moldboard plowing to a depth of 0.30 m in the summer,
114 followed by one or two shallow harrowing operations before planting. In the RT plots, primary

115 tillage was chisel plowing to a depth of 0.40 m (noninverting action) in the summer followed by
116 moldboard plowing to a depth of 0.15 m (after the first rains of autumn) and followed by one
117 shallow harrowing operation to prepare a proper seedbed; the moldboard plowing operation was
118 omitted beginning with the eighth year of the experiment (1998–1999). Finally, NT consisted of
119 sowing by direct drilling. The plot size was 370 m² (18.5 × 20.0 m). Each year, both rotations (W_F
120 and W_B) were duplicated in reverse order so as to obtain data annually from all crops. In NT plots,
121 weeds were controlled before planting with glyphosate (*N*-[phosphonomethyl] glycine) at a dose of
122 533–1066 g a.e. ha⁻¹, depending on the development of weeds.

123 During the wheat growing season, weeds were controlled by applying post-emergence herbicides
124 (varying the active ingredient during the experimental period) at the early growth stage of the crop,
125 with no differences among the three tillage systems. During the faba bean growing season, weeds
126 were controlled mechanically by shallow hoeing (with minimum soil disturbance) when the faba
127 bean plants were at the third-leaf stage; if necessary, the operation was repeated at the seventh-leaf
128 stage. During the berseem clover growing season, weeds were controlled by cutting all plants
129 (berseem clover and weeds) to an ~8-cm stubble height when the berseem clover had basal shoots
130 ~5 cm long. More details on how the trial was performed are reported in Giambalvo et al. [20] and
131 Amato et al. [21].

132

133 *Seedbank sampling and analysis*

134 Sampling of the weed seedbank was carried out at the end of two growing seasons, August 2007–
135 2008 and August 2008–2009 (the 17th and 18th years, respectively, after the beginning the
136 experiment) in the NT and CT treatments only. Each year, sampling was carried out only in the
137 plots where wheat was grown; this means that for W_W sampling was done each year at the same
138 plot, whereas for W_F and W_B sampling was done at both plots used for the rotations.

139 In both years, eight soil cores of 30 cm depth were randomly taken in each plot using a 5-cm-
140 diameter manual steel probe. Soon after the sampling, each core was subdivided into three subcores

141 corresponding to 0–5, 5–15, and 15–30 cm soil layers. Separately for each layer, the eight subcores
142 were pooled, and from each pool two subcores were extracted. Soil samples were kept in a dark
143 room at 4°C until processing. A total of 144 soil samples were used for the weed seedbank analysis.
144 The analysis was made using the seedling emergence method [10] by placing each soil sample in a
145 tray (30 × 20 × 5 cm) over a 2-cm-thick layer of coarse sand that had previously been sterilized in
146 an oven at 105°C for 72 h. A dense mesh was placed in between soil and sand to avoid mixing and
147 to facilitate periodic soil stirring. Trays were placed in a cold greenhouse for 12 months starting at
148 the end of November of each year, and they were watered regularly by sprinkler irrigation. To favor
149 dormancy breakdown, irrigation was suspended after 6 months for a period of 15 d, after which soil
150 samples were stirred. Weed seedlings that emerged were identified and counted by species at
151 regular time intervals and then removed. The classification of weed species into biological and
152 ecophysiological groups, for life cycle, and for type of seed dispersal was made according to Zanin
153 et al. [14] and Bärberi & Lo Cascio [10].

154

155 *Calculations and data analysis*

156 Weed communities under the different treatments (tillage techniques and crop sequences) were
157 compared using species richness, Shannon’s diversity index (H_{SH}), and Shannon’s evenness index
158 (E_{SH}). H_{SH} was calculated as follows:

159

$$160 H_{SH} = - \sum_{i=1}^S P_i \ln P_i ,$$

161

162 where

163

$$164 P_i = \frac{N_i}{N_{total}},$$

165

166 where N_i is the number of individuals of species i , N_{total} is the total number of individuals per soil
167 sample, and S is the total number of species found. Subsequently E_{SH} was calculated using the
168 following equation:

169

$$170 \quad E_{SH} = \frac{H_{SH}}{\ln S}.$$

171

172 Data from each year were analyzed separately for each soil layer, and the homogeneity of
173 variances was assessed using Bartlett's test before combined analyses were performed. Data can be
174 considered as coming from a split strip plot design, with time (random) as a whole plot and tillage
175 system (vertical) and crop sequence (horizontal) as a strip plot with two replicates. According to
176 Schabenberger & Pierce [22], the linear model to analyze such data contains four different
177 experimental error sources of variability, associated with the plot, the columns, the rows and their
178 intersection. In Table S1 sources of variability and degrees of freedom for a single soil layer are
179 reported. Analysis was carried out in the R environment [23]. Moreover, a canonical discriminant
180 analysis (CDA) was performed [24] using data on the 15 primary weeds detected to establish the
181 importance of each weed species in discriminating among the six cropping systems (combinations
182 of the two tillage systems and the three crop sequences). Canonical variable means (centroid values)
183 were calculated for each tillage system/crop sequence combination, and the significance between
184 means was determined using the Mahalanobis distance. Many studies have used CDA to
185 discriminate weed communities developing in different cropping systems [1,10,25].

186

187 **Results**

188 Type of dispersal, ecophysiological and biological groups, and relative density in the total
189 seedbank of all weeds are shown in Table 1. A total of 46 species were detected during the study,
190 72% of which were annuals, 9% biennials, and 19% perennials. Tillage system significantly

191 affected the number of weed species in different ways depending on crop sequence (Table 2). For
192 instance, in the upper soil layer (0–5 cm), the number of weed species detected was higher in NT
193 than in CT, with differences between these two tillage systems higher under W_W and W_B than W_F .
194 The opposite was true in the lower soil layer sampled (15–30 cm). In each layer (0–5, 5–15, 15–30,
195 and 0–30 cm), total weed seedling density differed significantly among the three crop sequences in
196 the order $W_W > W_F > W_B$ (Table 3). No variation was observed due to tillage system in the whole
197 layer sampled (0–30 cm); however, in both the upper and the intermediate layers, weed seedling
198 density was significantly higher in NT than CT, whereas in the lower layer the opposite was true.
199 The differences between CT and NT in both the upper and the lower layers were higher in W_W than
200 in W_F or W_B (the crop sequence \times tillage system interactions were significant at $P < 0.01$).

201 Table 4 reports total weed seedling density by biological group. Crop sequence significantly
202 affected total weed seedling density in the therophytes group, with values higher in W_W than W_F or
203 W_B (in the order $W_W > W_F > W_B$). The densities of hemicryptophytes varied by tillage system
204 (higher in CT than in NT, on average), whereas significant interactions were found between tillage
205 system and crop sequence for the densities of both biennials and geophytes (higher in NT than in
206 CT under W_W and W_B but not under W_F in biennials, and higher in NT than in CT only under W_F in
207 geophytes). No effect of tillage system was found for therophytes.

208 As regards ecophysiological group, only the density of species emerging (or sprouting) during
209 winter/spring and spring (i.e., by far the more abundant species) was affected by crop sequence (in
210 the order $W_W > W_F > W_B$; Table 5). Also, tillage system affected total weed seedling density for
211 spring and indifferent ecophysiological groups only; for both groups, the density was higher in NT
212 than in CT. For the spring/summer species, total weed seedling density was higher in NT than in CT
213 under W_F and W_B but not under W_W ; for the autumn species, it was higher in NT than in CT under
214 W_W , whereas the opposite was true under both W_F and W_B .

215 Table 6 shows the seedling density of the primary weed species, which together accounted for
216 almost 90% of the total weed seedlings, regardless of the treatment applied. For most of these

217 species, the effect of tillage system on the total seedling density varied significantly by crop
218 sequence. For instance, the seedling density of *Polygonum aviculare* was higher in CT than in NT,
219 with differences between these two tillage systems higher under W_W than W_F or W_B . The opposite
220 was true for *Papaver rhoeas*. The total seedling densities of *Lactuca serriola* and *Lolium* spp. were
221 higher in NT than in CT under W_B and W_W but not under W_F . The total density of seedlings for
222 *Anagallis arvensis*, was significantly affected by crop sequence (in the order $W_W > W_F > W_B$) and
223 tillage system (NT > CT). Both H_{SH} and E_{SH} were significantly affected by crop sequence, being
224 lower in W_W than in W_F or W_B (Fig. 1), whereas no effect of tillage system was found on these
225 indices.

226 The CDA based on data on seedling density of the major weed species clearly discriminated the six
227 cropping systems (Fig. 2). The first two canonical variables accounted for about 75% of the total
228 variance, which can be considered adequate for a bi-dimensional representation. CAN1 accounted
229 for 53.6% of the total variance and was positively influenced by *Phalaris* spp., *P. rhoeas*, *Veronica*
230 *hederifolia*, *L. serriola*, *Lolium* spp., and *Chenopodium vulvaria*. CAN2 explained 22.4% of the
231 variance; the weed species with the greatest influence were *Sonchus asper* and *P. rhoeas* (both
232 positive) and *Anagallis arvensis* and *Portulaca oleracea* (both negative). CAN1 separated NT- W_W
233 and NT- W_F cropping systems from CT- W_B and NT- W_B , while CAN2 separated CT- W_W from CT-
234 W_F .

235

236 Discussion

237 Tillage system and crop sequence interacted to determine weed species richness. Regarding this,
238 contradictory results can be found in the literature. For instance, Dorado et al. [5] and Sosnoskie et
239 al. [1] observed greater weed species richness in crop rotations compared to monocultures and when
240 the tillage intensity decreased, whereas Fried et al. [26] found a higher number of weed species in
241 deeply tilled fields compared to those in which NT or minimum tillage were applied. After 12 years
242 of application of four tillage systems in two crop rotations Barberi & Lo Cascio [10] found that the

243 number of weed species in the total seedbank did not substantially vary among treatments. They
244 argued that although management practices (such as tillage system and crop rotation) can exert a
245 considerable effect on the emergence and growth of weed species, they can have little or no effect
246 on the reserve of biodiversity in the soil, mainly because of the seed longevity of many weed
247 species, which can serve as a buffer against environmental variability to reduce the risk of
248 extinction. The discrepancy in the aforementioned results shows that the impact of different soil
249 tillage techniques and crop sequences on weed species richness is likely to be highly site specific;
250 this is not surprising given the intrinsic variability in climatic conditions, soil characteristics,
251 management practices, agronomic history, and duration of the experiments. As concerns our
252 experiment, we found that the continuous use of NT, compared to CT, led to a reduction in the
253 number of weed species only under W_W and W_F , whereas under W_B no differences between CT and
254 NT were observed. It is likely that the weed control strategies applied in W_B , based on a spring cut
255 of the vegetation before dissemination of weeds occurred, could have masked the effects of tillage
256 system. As regards the diversity indices, both H_{SH} and E_{SH} were significantly affected by crop
257 sequence, being higher in the two-crop rotations than in the wheat monoculture. This result is in
258 agreement with the findings of Légère et al. [8] and Sosnokie et al. [1]. The crop sequences together
259 with their associated cultural practices (sowing time, weed management strategies, fertilization,
260 etc.) modified the physical, chemical, and biological conditions of the soil, which may have
261 differentially influenced the emergence, growth, and capacity of species to produce seeds, thus
262 modifying their relative abundance. The fact that crop rotations affect the abundance of certain
263 weed species (including some species that are particularly problematic, such as *P. aviculare*)
264 suggests that such agronomic practice can be essential when planning efficient weed control
265 strategies. As concerns tillage system, although it modified the weed composition by altering the
266 relative abundance of many species, it did not influence either H_{SH} or E_{SH} . This result is in line with
267 the findings of Légère et al. [8], who observed that tillage had little effect on weed diversity indices
268 but played an important role in determining the composition of the weed community.

269 Total weed seedling density was significantly influenced by crop sequence (in the order $W_W >$
270 $W_F > W_B$). The introduction of berseem clover in the crop sequence resulted in a dramatic reduction
271 in potential weed infestation; this result can be explained by the positive effects of disturbance
272 caused by the diversification and, specifically, the fact that the seed yield of berseem clover is
273 obtained from regrowth after a spring cut, before dissemination of weeds occurs. The spring cut,
274 together with the excellent regrowth ability of berseem clover [27,28], greatly limits the possibility
275 that weed will produce seeds. The reduction in total seed weed density detected in W_F compared to
276 W_W is more difficult to explain considering that compared with wheat, faba bean has a sparser
277 canopy (as a result of a greater row spacing) and a slower growth rate in the early stages of the crop
278 cycle, both characteristics that favor the emergence and growth of weeds [29]. Moreover, during the
279 faba bean growing season, weeds were controlled mechanically by shallow hoeing, which did not
280 always guarantee an optimal result [20]. It is likely that the positive effects of disturbance caused by
281 the diversification of the cropping system (faba bean–wheat rotation vs. continuous wheat) widely
282 counteracted the negative effect due to the increased weed dissemination during the faba bean
283 phase.

284 Tillage system did not affect total weed seedling density but markedly influenced the distribution
285 of weed seeds along the soil profile. According to Ball [4], NT left a greater proportion of seeds
286 near the soil surface (particularly evident in W_W), whereas in CT weed seeds were more or less
287 uniformly distributed along the tillage layer.

288 Both tillage system and crop sequence led to qualitative changes in weed flora. The continuous
289 use of NT led to an increase in eight species—*P. rhoeas*, *A. arvensis*, *V. hederifolia*, *L. serriola*,
290 *Phalaris* spp., *Lolium* spp., *E. elaterium*, and *P. oleracea*—even if, for most of these species, the
291 magnitude of the differences between the two tillage systems was markedly affected by crop
292 sequence. Other authors have found a progressive increase in most of these species due to a
293 reduction in tillage intensity [5,10]. These species differ markedly in their ability to compete with
294 crops; for instance, *A. arvensis* is not aggressive [30], whereas *L. serriola* is particularly competitive

295 and thus able to cause considerable losses in yield. From an agronomic point of view, the increased
296 abundance of this species represents a serious weed management concern for NT cropping systems.
297 For many species, particularly for *P. rhoeas*, the superiority of NT over CT (in terms of seedling
298 density) was less under W_B with compared to both W_W and W_F . This result is probably attributable
299 to the weed control strategy adopted during the berseem clover growing season (i.e., the spring cut
300 of the vegetation) which drastically reduced the probability of seed dissemination for many weed
301 species, thus masking the effect of tillage system in the W_B crop sequence. The severe reduction in
302 seedling density of many weed species in W_B probably led to a release of ecological niches that
303 were then occupied by other species. This may have been true for both *P. oleracea* and *V.*
304 *hederifolia*, whose density increased only in the NT- W_B system. This result can be explained
305 considering the growth habit of these two species (which is prostrate or semi-prostrate), a trait
306 which probably allowed plants to avoid the cut made during the berseem clover growing season,
307 thus increasing the probability of their seeds being disseminated. As concerns monocotyledons, the
308 increases in seedling density observed for *Phalaris* spp. and *Lolium* spp. in NT systems were
309 particularly pronounced under W_W . These two species are favored by the cessation of weed control
310 by superficial soil disturbance [14,31,32]. Moreover, in our experiment, the 18-year continuous
311 application of wheat monoculture resulted in conditions favorable to these two species also due to
312 the use of herbicides on wheat, which are less efficient on monocotyledons than on eudicotyledons
313 [33]. With regard to *Phalaris* spp., other studies have found opposite results from ours, underlining
314 a higher density of *Phalaris* spp. in CT than in conservative tillage systems [34,35]. Taylor et al.
315 [36] found that peak emergence of *Phalaris paradoxa* seedlings was in the middle of the winter
316 cropping season in NT plots but at the beginning of the cropping season in cultivated plots. This
317 altered periodicity of the emergence of *Phalaris* spp. seedlings can make it easier to control
318 seedlings in CT plots through pre-sowing operations; this in turn could lead to a progressive decline
319 in *Phalaris* spp. seeds in the seedbank compared with plots that receive no cultivation. In our
320 experiment, *P. aviculare* showed a preference for moldboard plowed soil, a result that is in

321 agreement with Dorado & López-Fando [34]. For this species germination is markedly affected by
322 both fluctuations in soil moisture conditions experienced by seed during dormancy and the
323 sensitivity of seed to light [37], both factors that are modified by tillage system. As regards *R.*
324 *segetum* and *S. arvensis*, an increase in viable seeds in CT compared with NT was observed in the
325 W_F crop sequence only. Other authors have highlighted a preference of *S. arvensis* for moldboard
326 plowed soil [7], whereas no information is available for *R. segetum*. In the present experiment, the
327 differences in seedling density between CT and NT as the crop sequence changed are attributable to
328 the different weed control methods adopted in the three crop sequences. The spring cut of berseem
329 clover and the herbicides applied on wheat effectively controlled both *R. segetum* and *S. arvensis*,
330 whereas mechanical control (shallow hoeing) performed during the faba bean growing season did
331 not always guarantee efficient control, thus allowing these two species to disseminate.

332 According to many authors [1,5,7], crop sequence markedly influences the weed seedbank in
333 both quantitative and qualitative terms by creating environmental conditions that differentially
334 affect species emergence, development, and dissemination. Some species, such as *P. aviculare* and
335 *A. arvensis*, were markedly more abundant in W_W than in W_F or W_B , whereas, as mentioned
336 previously, the seedling density of *P. rhoeas* was lower in W_B than in W_W or W_F . According to
337 Légère & Samson [38] and Menalled et al. [39], crop sequence effects cannot be distinguished from
338 associated cultural practices or, in particular, from the effects of weed control strategies that, in our
339 study, differed widely in relation to the crop species.

340 The CDA allowed us to discriminate among the different cropping systems, highlighting how the
341 interaction of the treatments applied (tillage system and crop sequence) affected the weed seedbank
342 in both quantitative and qualitative terms. The presence of berseem clover in the crop rotation
343 markedly influenced the composition of weed community, masking, at the same time, the effects of
344 tillage system. In contrast, NT exerted great pressure on weed communities in both the W_W and W_F
345 cropping systems, which were plotted near each other and distant from all other treatments, whereas
346 crop sequence (W_W vs W_F) differentiated weed community composition only under CT. Some

347 authors [13,14] have offered an ecological interpretation of weed flora dynamics under different
348 tillage systems. In particular, Zanin et al. [14] reported that a reduction in the mechanical
349 disturbance of soil can result in marked changes to flora, with a tendency for weeds to undergo
350 succession toward a more mature community, with an increased importance of biennial,
351 hemicryptophytes, and geophytes species. Other studies have highlighted the fact that a reduction in
352 soil disturbance generally results in an increase in the occurrence of perennial weeds in many arable
353 cropping systems [40,41]. Although biennial weed species were generally favored in NT systems in
354 our study, the data did not allow us to clearly demonstrate the existence of a gradient reflecting
355 ecological community succession. The effects of tillage system probably could have been reduced
356 or masked by other agronomic factors, as observed by Derksen et al. [42] and Légère et al. [8] in
357 other studies.

358 In conclusion, this weed seedbank analysis performed within a long-term (18-year) field
359 experiment has provided useful information about the effects of some agronomic practices on weed
360 population dynamics in wheat-based Mediterranean cropping systems. Our results suggest that crop
361 rotation and tillage technique both act as filters that determine (and, moreover, often interact with
362 each other in determining) the composition and abundance of weed species in the soil seedbank. In
363 particular, compared with crop rotations (wheat–faba bean and, particularly, wheat–berseem
364 clover), the continuous monoculture of wheat resulted in a great increase in total weed seedbank
365 density and, at the same time, a reduction in weed diversity, with a strong increase in some species,
366 some of which are potentially hard to control. In contrast, tillage system had no effect on the size of
367 the weed seedbank but significantly modified its composition as well as the distribution of weed
368 seeds along the soil profile. Indeed, the adoption of a CT technique (based on moldboard plowing)
369 favored some weed species (mainly *P. aviculare*); in contrast, the continuous use of NT led to an
370 increase in weed seeds in the upper soil layer and, moreover, resulted in a significant increase in the
371 seed density of some problematic species, such as *P. rhoeas*, *Phalaris* spp., and *L. serriola*. In any
372 case, the effects of tillage system on weed seedbank size and composition were enhanced in both

373 the wheat–faba bean cropping system and the continuous monoculture of wheat but weakened in the
374 wheat–berseem clover cropping system. Hence, knowledge on how agricultural practices influence
375 the weed community in the mid and long term, from both a quantitative and qualitative point of
376 view, may enable experts to predict the spread of problematic weeds and to plan efficient control
377 strategies that favor the development of a weed community with little impact on the agroecosystem.
378 From a practical point of view, these results suggest that, although NT is environmentally friendly
379 because it mitigates soil erosion, reduces energy use, and enhances wildlife habitat, farmers should
380 only apply such a conservative technique within an appropriate crop sequence.

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494 **Figures legends**

495 **Figure 1. Shannon's diversity index (H_{SH}) and Shannon's evenness index (E_{SH}) in the six**
496 **cropping systems.** CT, conventional tillage; NT, no tillage; W_W , continuous wheat; W_F , wheat–
497 faba bean; and W_B , wheat–berseem clover. CS, cropping sequence; TS, tillage system.

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501 **Figure 2. Canonical discriminant analysis ordination biplot of the six cropping system**
502 **centroids.** CAN1, first canonical variable; CAN2, second canonical variable. CT, conventional
503 tillage; NT, no tillage; W_W , continuous wheat; W_F , wheat–faba bean; and W_B , wheat–berseem
504 clover. The direction and length of each line indicates the degree of association between each weed
505 species and cropping system. Only the 15 primary weeds are displayed. Pr, *Papaver rhoeas*; Aa,
506 *Anagallis arvensis*; Pa, *Polygonum aviculare*; Vh, *Veronica hederifolia*; Ls, *Lactuca serriola*; Ph,
507 *Phalaris* spp.; Cv, *Chenopodium vulvaria*; Ee, *Ecballium elaterium*; Lo, *Lolium* spp.; Sas, *Sonchus*
508 *asper*; Dt, *Diploaxis tenuifolia*; Sm, *Stellaria media*; Po, *Portulaca oleracea*; Sar, *Sinapis*
509 *arvensis*; Rs, *Ridolfia segetum*. Comparisons of Mahalanobis squared distances showed highly
510 significant differences ($P < 0.01$) in the compositions of weed communities among all cropping
511 systems.

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516 **Table 1.** Weed populations in the six cropping systems (CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and
 517 W_B, wheat–berseem clover) classified into biological group, ecophysiological group, life cycle, and type of dispersal.

Number	Species	Biological group	Ecophysiological group	Life cycle	Type of dispersal	Relative density (%)	W _W		W _F		W _B	
							CT	NT	CT	NT	CT	NT
1	<i>Ammi majus</i>	Th	Sp	An	Baro	0.37	+	+	+			+
2	<i>Anagallis arvensis</i>	Th	Sp	An	Baro	24.86	+	+	+	+		+
3	<i>Brassica juncea</i>	Th	Au	An	Baro	0.10			+	+		+
4	<i>Bromus hordeaceus</i>	Th	Au	An	Zooc/Anem	0.45	+	+	+			+
5	<i>Campanula erinus</i>	Th	Au	An	Baro	0.23	+					+
6	<i>Capsella bursa-pastoris</i>	H2	In	Bi	Baro	0.16		+	+			
7	<i>Chenopodium album</i>	Th	Au	An	Baro	0.06	+	+	+	+		+
8	<i>Chenopodium vulvaria</i>	Th	Sp	An	Baro	2.28	+		+			
9	<i>Cirsium arvense</i>	G	Sp	Per	Anem	0.10			+	+		+
10	<i>Convolvulus</i> spp.	G	Sp	Per	Baro	0.53	+	+	+	+		+
11	<i>Digitaria sanguinalis</i>	Th	Su	An	Baro	0.04		+	+			
12	<i>Diploaxis tenuifolia</i>	Hr	Wi/Sp	Per	Baro	1.43	+	+	+	+		+
13	<i>Dittrichia viscosa</i>	Hr	–	Per	Anem	0.03						+
14	<i>Ecballium elaterium</i>	G	Sp/Su	Per	Baro	2.31	+	+	+	+		+
15	<i>Festuca arundinacea</i>	Hr	Au	Per	Baro	0.14	+		+	+		
16	<i>Galactites tomentosa</i>	H2	Au/Wi	Bi	Anem	0.11	+		+	+		+
17	<i>Galium aparine</i>	Th	Au	An	Baro	0.17	+	+				
18	<i>Heliotropium aeuropium</i>	Th	Su	An	Baro	0.78	+	+	+	+		+
19	<i>Hordeum murinum</i>	Th	Au	An	Baro	0.01						+
20	<i>Kickxia spuria</i>	Th	Sp	An	Baro	0.27	+			+		+
21	<i>Lactuca serriola</i>	H2	Sp	Bi	Anem	2.61	+	+	+	+		+
22	<i>Lamium purpureum</i>	Th	In	An	Baro	0.22	+		+	+		+
23	<i>Linaria chalepensis</i>	Th	–	An	–	0.10			+	+		+
24	<i>Lolium</i> spp.	Th	In	An	Baro	1.88	+	+	+	+		+
25	<i>Lythrum junceum</i>	Hr	–	Per	–	0.79	+		+			+
26	<i>Malva nicaeensis</i>	H2	In	Bi	–	0.02		+				
27	<i>Mercurialis annua</i>	Th	Su	An	Baro	0.14	+					
28	<i>Oxalis pes-caprae</i>	G	Au/Wi	Per	–	0.07						+
29	<i>Papaver rhoeas</i>	Th	Wi	An	Baro	26.91	+	+	+	+		+
30	<i>Phalaris</i> spp.	Th	Au	An	Baro	2.20	+	+	+	+		+
31	<i>Polygonum aviculare</i>	Th	Wi/Sp	An	Baro	19.16	+	+	+	+		+
32	<i>Portulaca oleracea</i>	Th	Sp/Su	An	Baro	1.10		+	+	+		+
33	<i>Raphanus raphanistrum</i>	Th	Sp	An	Baro	0.06			+	+		
34	<i>Ridolfia segetum</i>	Th	Wi/Sp	An	Baro	0.84	+	+	+	+		+
35	<i>Scandix pecten-veneris</i>	Th	In	An	Baro	0.11	+	+	+			+
36	<i>Senecio vulgaris</i>	Th	In	An	Anem	0.44	+	+	+	+		+
37	<i>Sinapis arvensis</i>	Th	Au/Wi	An	Baro	1.11	+	+	+	+		+
38	<i>Solanum nigrum</i>	Th	Sp/Su	An	Zooc	0.03						+
39	<i>Sonchus asper</i>	Th	In	An	Anem	1.86	+	+	+	+		+
40	<i>Sonchus oleraceus</i>	Th	In	An	Anem	0.54	+	+	+	+		+
41	<i>Stellaria media</i>	Th	In	An	Baro	1.28	+	+	+	+		+
42	<i>Taraxacum officinalis</i>	Hr	In	Per	Anem	0.08	+	+		+		+
43	<i>Trifolium</i> spp.	Th	Au/Wi	An	Baro	0.18	+	+		+		+
44	<i>Triticum durum</i>	Th	Au/Wi	An	Baro	1.10	+	+	+	+		+
45	<i>Veronica hederifolia</i>	Th	In	An	Baro	2.43	+	+	+	+		+

46	<i>Veronica persica</i>	Th	In	An	Baro	0.33	+	+	+	+
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518 Th, therophytes; H2, biennial species; G, geophytes; Hr, hemicryptophytes. Au, Au/Wi, Wi/Sp, Sp, Sp/Su, Su: autumn, autumn/winter, winter/spring, spring, spring/summer,
519 summer germinating species. In, indifferent (species germinating in any month). An, annual species; Bi, biennial species; Per, perennial species. Baro, barochory; Anem,
520 anemochory; Zooc, zoochory; Zooc/Anem, zoochory/anemochory.

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Table 2. Number of weed species detected in each soil layer.

Soil layer (cm)	W _W		W _F		W _B		P-value		
	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
0–5	15.5	18.0	17.5	18.0	15.0	17.5	0.866	0.101	0.045
5–15	13.5	13.5	13.0	12.0	12.5	13.0	0.528	0.187	0.009
15–30	16.5	12.0	16.5	9.0	14.0	11.5	0.251	0.074	0.035
0–30	25.0	22.5	27.5	22.5	22.0	23.0	0.479	0.660	0.004

528 CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover.
529 CS, crop sequence; TS, tillage system.

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Table 3. Weed seedling density (number of seedlings per kilogram of dried soil) detected in each soil layer in the six cropping systems.

Soil layer (cm)	W _W		W _F		W _B		P-value		
	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
0–5	32	139	32	85	17	41	< 0.001	< 0.001	0.003
5–15	28	40	23	36	11	21	0.002	0.001	0.757
15–30	66	19	27	13	17	11	< 0.001	0.020	0.001
0–30	48	44	26	31	15	19	< 0.001	0.630	0.187

537 CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover.
 538 CS, crop sequence; TS, tillage system.

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Table 4. Total weed seedling density (number of seedlings per kilogram of dried soil) by biological group detected in the six cropping systems.

	W _W		W _F		W _B		P-value		
	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
Therophytes	46.2	41.5	23.0	27.3	12.8	16.8	< 0.001	0.709	0.182
Biennial species	0.4	1.5	0.8	1.0	0.3	1.3	0.822	0.027	0.016
Hemicryptophytes	0.9	0.3	1.7	0.1	1.0	0.5	0.307	0.039	0.091
Geophytes	0.6	0.6	0.8	2.5	0.7	0.4	0.046	0.063	0.004

546 CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover.
 547 CS, crop sequence; TS, tillage system.

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Table 5. Total weed seedling density (number of seedlings per kilogram of dried soil) by ecophysiological group detected in the six cropping systems.

	W _W		W _F		W _B		P-value		
	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
Autumn	1.1	1.9	1.1	0.6	1.1	0.3	0.143	0.796	0.005
Autumn/Winter	0.2	0.5	2.0	1.1	0.6	0.3	0.142	0.537	0.429
Winter/Spring	29.5	18.1	13.3	15.7	8.9	2.9	< 0.001	0.121	0.055
Spring	13.4	18.3	5.7	9.3	1.8	8.4	< 0.001	0.001	0.216
Spring/Summer	0.5	0.4	0.5	2.5	0.4	2.0	0.020	0.026	0.007
Summer	0.4	0.3	0.1	0.0	0.2	0.7	0.196	0.325	0.087
Indifferent	2.9	4.4	2.3	1.6	1.8	4.2	0.124	0.034	0.058

555 CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover.
 556 CS, crop sequence; TS, tillage system.

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Table 6. Total seedling density (number of seedlings per kilogram of dried soil) for the 15 primary weed species detected in the six cropping systems.

Species	W _W		W _F		W _B		P-value		
	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
<i>Anagallis arvensis</i>	11.9	16.2	2.0	7.8	1.1	6.5	< 0.001	0.001	0.700
<i>Chenopodium vulvaria</i>	0.8	0.1	2.4	0.6	0.0	0.2	0.054	0.018	0.059
<i>Diploaxis tenuifolia</i>	0.6	0.3	0.2	0.1	1.0	0.4	0.053	0.336	0.443
<i>Ecballium elaterium</i>	0.5	0.3	0.4	2.4	0.4	0.2	0.018	0.007	0.003
<i>Lactuca serriola</i>	0.3	1.4	0.8	0.8	0.2	1.3	0.858	0.024	0.004
<i>Lolium</i> spp.	0.2	1.9	0.1	0.1	0.4	0.8	0.013	0.014	0.004
<i>Papaver rhoeas</i>	5.1	16.0	10.0	15.2	0.8	2.1	0.003	0.007	0.019
<i>Phalaris</i> spp.	0.4	1.7	0.4	0.6	0.7	1.2	0.019	0.215	< 0.001
<i>Polygonum aviculare</i>	23.4	1.5	2.5	0.3	7.1	0.3	< 0.001	0.004	< 0.001
<i>Portulaca oleracea</i>	0.0	0.1	0.0	0.1	0.0	1.8	0.016	0.055	0.014
<i>Ridolfia segetum</i>	0.3	0.3	1.0	0.1	0.0	0.1	0.154	0.109	0.040
<i>Sinapis arvensis</i>	0.0	0.3	1.3	0.0	0.3	0.0	0.113	0.129	0.019
<i>Sonchus asper</i>	0.6	0.6	0.9	0.4	0.4	0.5	0.667	0.138	0.146
<i>Stellaria media</i>	1.1	0.6	0.0	0.2	0.2	0.2	0.233	0.212	0.526
<i>Veronica hederifolia</i>	0.5	0.9	0.1	0.4	0.0	2.5	0.021	0.019	0.039

564 CT, conventional tillage; NT, no tillage; W_W, continuous wheat; W_F, wheat–faba bean; and W_B, wheat–berseem clover.
565 CS, crop sequence; TS, tillage system.

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