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Stale seedbed preparation for sustainable weed seed bank management in organic cropping systems

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Abstract

Stale seedbed preparation is a neglected agronomic strategy used to decrease weed seed banks. The aim of the experiments was to verify the quanti-qualitative seed bank reduction after different soil tillage typologies: rotary cultivator, rotary harrowing and spike tooth harrowing (tillage depth in each case uniformed to about 15 cm). Tillage was carried out during the spring-summer period, with five tillage sequences spaced about 30-40 days. The weed seedbank analysis (10-30 cm) showed that beyond a 10 cm soil depth, the buried seeds were unaffected irrespective of the kind of soil tillage since no seed depletion was observed. In contrast, weed seed bank was heavily depleted in the shallowest soil layer (0-10 cm) due to the germination trigger induced by the soil aeration and by the consequent increase of oxygen availability after tillage. This seed bank reduction, was proportional to the degree of soil crumbling induced by the different tillage methods and it was higher in the case of the smaller soil clods size. Each weed species showed the highest emergence dynamics when soil tillage was carried out during the periods most suitable to meet the respective thermal requirements. Indeed the earliest soil tillage in April triggered germination and emergence of microthermal weeds, while those carried out in May and June triggered the emergence dynamics of weeds characterized by higher thermal requirements. The emergence rate, after

26 the stale seedbed preparation, showed high values overall in the case of deep soil crumbling. In addition
27 the extent of soil crumbling was positively related to the biodiversity of the emerged weed communities.
28 The weed species that were the least sensitive to stale seedbed preparation were those characterized by
29 small seeds and consequently those species would be more difficult to reduce through stale seedbed
30 preparation.

31
32 **Running head:** Stale seedbed for weed control

33
34 **Keywords**

35 Weed seed; Seed burial; Weed emergence; Seed bank depletion, Soil tillage

36
37 **Highlights**

- 38 ► The degree of soil crumbling measured by clods size is proportional to the weed emergence rate.
- 39 ► A decrease in the seed bank occurs only within the shallowest soil layer (0-10 cm).
- 40 ► Greater soil crumbling allows the germination trigger of a higher number of species.
- 41 ► The smaller and lighter seeds show the lower emergence rate due to their greater burial intolerance.
- 42 ► Stale seedbed preparation sequences can play a crucial role in the weed management sustainability.

43
44 **Introduction**

45 Since the beginning of agriculture up to the Second World War, weed management was based on
46 preventive strategies, through appropriate agronomic practices (Altieri, 2004), capable of minimizing the
47 need for a curative crop protection. These historical cropping systems, today referred to as “sustainable”
48 (Wezel *et al.*, 2014), is an increasingly requirement to minimize the use of herbicides.

49 Unfortunately this agronomic simplification, which evolved in the post-war period during the so-called
50 “green revolution” (Evenson and Gollin, 2003), has made the crop protection more vulnerable by the

51 dominance of more aggressive weed species. Unfortunately the discovery of effective herbicides means
52 that preventive methods have been superseded by curative methods without looking for an integration
53 between them. Yet the use of herbicides alone is unlikely a decisive remedy and is only effective in the
54 long-term when the efficacy shown in a single year can be maintained for a long period thanks to an
55 integrated weed management (Swanton et al., 2008) using a wide range of agronomic practices.

56 The onset throughout the world of herbicide-resistant weeds (Heap, 2020), is a sort of “tip of the iceberg”
57 which springs from a rigid weed management not only in terms of the prolonged use of the same
58 herbicides but also of the extreme simplification of crop rotations and soil tillage (Powles, 2008). Today
59 one of the most requested agronomic innovations is thus based on the re-discovery of ancient agronomic
60 practices that make several cropping systems sustainable. In other words, in addition to the extreme case
61 of “organic” cropping systems, in which no synthetic herbicides are used, these ancient agronomic
62 practices should also be included in “conventional” cropping systems, allowing “integrated” cropping
63 systems capable to make the agricultural protection sustainable over time. An important way to allow
64 sustainable weed management is not to exert a agronomic pressure able to select oligo-or even
65 monospecific weed communities since the dominance of a few species implies a very difficult control
66 (Storkey and Neve, 2018). In other words, the biodiversity of the botanical structure of the weed
67 populations is an effective indicator of sustainability both from an ecological and agronomic point of view.
68 This objective can be achieved by integrating appropriate agronomic practices (crop rotation, tillage, cover
69 crops, etc.) with curative and preventive control methods in a context of low-intensity farming.

70 The extreme scarcity of effective curative methods in sustainable agricultural systems makes preventive
71 operations even more crucial (Pannacci et al., 2017). In fact, the greatest critical issue in the economic
72 sustainability of organic agriculture is due to the extreme abundance of weed populations, resulting in a
73 substantial drop in crop yields (Seufert et al., 2012). Except for unusual and specific eco-compatible
74 methods (Li et al., 2012), these infestations are difficult to manage in the long term in organic cropping

75 systems due to the heavy "seed rain". This leads to the accumulation of a considerable amounts of seeds in
76 the soil for both arable (Teasdale et al., 2004) and horticultural (Benvenuti and Pardossi, 2017) crops.

77 One of the most important strategies to prevent weed control, which unfortunately is rarely used, is the
78 stale seedbed preparation, also called false seedbed preparation, which consists of one, or more, seedbed
79 preparations, not followed by crop sowing, that trigger weed seed bank germination. The emerged weed
80 seedlings are then eliminated with subsequent agronomic disturbances often carried out mechanically
81 (Rasmussen, 2004). In fact the seedbed preparation triggers germination (Boyd et al., 2006) of part of the
82 weed seed bank when it is exposed to limiting-factors for seed germination such as oxygen light and seed-
83 soil contact in the case of weed seeds placed on the soil surface (Gardarin et al., 2011). In this context the
84 greatest obstacle to weed seed germination is given by the micro-environment that surrounds the seeds.
85 Indeed the soil particles, overall when they are aggregated into clods, play a crucial role in allowing weed
86 seed bank accumulation due to physical constraints (Benvenuti and Mazzoncini, 2019), which is why most
87 weeds in the agro-ecosystem are in a "latent" state as seeds in the soil waiting to "wake up" and invade
88 the crop. This long-term (Burnside et al., 1996) latent life is due to: i) frequent seed dormancy, both
89 physical and/or physiological (Baskin and Baskin, 2004), and ii) the scarcity and/or lack of the ecological
90 factors needed for germination, such as oxygen and light during the hydrothermal period (Masin et al.,
91 2012). Every year only a small part of this seed bank germinates (sometimes even less than 1%, Forcella et
92 al., 1992) thus keeping most of the viable seeds in a quiescent and/or dormant state and thus capable of a
93 cyclic re-invasion of the agroecosystem.

94 The agronomic "forcing" of buried weed seed germination is the main agronomic strategy used to deplete
95 the seedbank. Unfortunately this is hindered by the typical physiological (Vleeshouwers et al., 1995),
96 physical (Paulsen et al., 2013) and/or environment-mediated (Benech-Arnold et al., 2000) seed dormancy.

97 The last kind of dormancy is called secondary dormancy (Hilhorst, 1998).

98 After loss of dormancy (Allen and Meyer, 1998), seeds undergo a cyclical dormancy re-induction (Karsen,
99 1980) due to the external ecological burial conditions caused by: i) excessive depth (Benvenuti et al., 2001);

100 ii) physical soil ecology in terms of clod size, compaction, surface crust, limited gaseous diffusion typically
101 occurring in silty and/or clayey soils (Cussans et al., 1996); and iii) flooding (Mollard et al., 2007). Repeated
102 cycles of seedbed preparation are an important agronomic strategy since they break dormancy and trigger
103 weed seed germination, thus decreasing the seed bank and the subsequent potential for crop invasion.
104 This seedbed preparation can be carried out using different tools, both not rotating (spike tooth harrow)
105 and rotating vertically (rotary cultivator), or horizontally (rotary harrow). Each of these tools involves a
106 different physical action on the soil aggregates in terms of softness, aeration, and size.

107 Despite the growing agronomic importance of stale seedbed preparation, especially in the case of organic
108 farming systems, there is little information on the modalities (times and tools) that optimize these
109 operations .

110 The purpose of our experiment was: i) to quantify the weed seed bank depletion after different methods of
111 stale seedbed preparation; ii) to verify the periods of greatest effectiveness on the basis of the prevalent
112 weed species; iii) to evaluate the performance of the weed seed bank depletion in the various soil layers;
113 and iv) find a relationship between the efficacy of the “forced” field seedling emergence of various weeds
114 and their respective seed traits.

116 **Material and methods**

117 *Agronomic environment*

118 The experiments were carried out in 2015 in Tuscany near Sansepolcro, (Italy, 43° 36' North, 10° 20' East)
119 at the Aboca Farm specialized in the production and processing of medicinal herbs using organic cropping
120 systems. The experimental area (roughly 10 ha) was selected due to its uniformity of management in terms
121 of soil texture and previous agronomic practices. In the last 10 years the following species had been
122 rotated: Chamomile (*Matricaria chamomilla* L.), Purple Coneflower (*Echinacea purpurea* L.), Mallow (*Malva*
123 *sylvestris* L.), Passionflower (*Passiflora incarnata*), and Dandelion (*Taraxacum officinale* L.). Throughout the
124 10-year period, the same tillage techniques had been used: ploughing to 25 cm and using disk harrow for

125 seedbed preparation. This area is also characterized by a marked uniformity in terms of both: i) pedologic
126 characteristics (USDA classified xerofluent loam soil, 65% sand, 20% lime, 15% clay; pH 7.2, 1.8 organic
127 matter); and ii) botanical structure and quantity of existing weed communities. In particular, it should be
128 noted that the previous rotation of medicinal crops (often characterized by multi-year agronomic cycle),
129 had selected weed communities of both: autumn-winter and spring-summer cycle.

130 As expected, during the experimental period, rain was rather scarce in the summer (especially in July)
131 although there were rains throughout the experimental period (about 80 mm in May, 70 in June, 40 in July,
132 50 in August and 65 in September, Figure 1). Thus there were no periods of drought that might otherwise
133 have compromised the weed germination and the relative field emergence dynamics. In addition the rain
134 did not prevent the regular performance of the planned soil tillage calendar.

135 136 *Previous experimental problems*

137 During the two years preceding this experiment (2013 and 2014) occurred agronomic problems due to the
138 high climatic requirements that this experimentation implies: no rains before the planned soil tillage
139 calendars. In fact, some rains that occurred during the spring and/or summer periods of both years (2013-
140 2014) prevented the necessary field trafficability due to the excessive soil humidity. Unfortunately, the
141 inevitable delays of the soil tillage sequence, compared to the expected calendar (monthly sequence),
142 allowed many emerged weeds to ripen a not negligible seed quantity with consequent seed dispersal.
143 Obviously this did not allow to correctly evaluate the decrease of the seed bank (initial and final). Only in
144 the third year did the more fortunate climatic conditions allow the planned experiments to be completed
145 without problems of field trafficability. Consequently, it is worth highlighting that this particular
146 experimental trials is very difficult to repeat over time.

147 148 *Stale seedbed preparation techniques*

149 During the year 2015 three stale seedbed management techniques were compared: i) rotary cultivator; ii)
150 spike tooth harrow, (iii) rotary harrow and iv) untilled control. Each type of soil tillage was carried out five
151 times with a 5-6 week gap in between following preliminary tests that showed the maximum degree of
152 seedling emergence within about a month of the soil tillage. Each soil tillage intervention was carried out
153 on the same days: 12 March, 21 April, 4 June, 27 July, 10 September. The depth of each of the three soil
154 tillage was uniformed to about 15 cm. During the expected periods of soil tillage, the water content was in
155 fact almost optimal (45-65%) throughout the selected periods (data not shown). In accordance with
156 previous findings carried out with similar loam soil (Mueller et al., 2003), this humidity is considered
157 optimal for soil tillage.

158 Four replicate plots (30 m × 120 m) for each seedbed management techniques were carried out. A
159 randomized block was adopted as the experimental design and the sequence of agronomic interventions
160 and the analyses of seed bank are chronologically shown in Figure 2 and visually in the Figure 3.

162 *Soil aggregate size evaluation*

163 Soil samples were collected after the tillage intervention of 4 June when the soil moisture conditions were
164 assessed as optimal for this evaluation. This sampling was carried out from a 0-10 cm layer in each plot
165 using a rectangular trough (15 cm x 17.5 cm) with minimal disturbance and samples were sealed in plastic
166 bags according to Kemper and Rosenau (1986). The soil was exposed to air dry for three days. Samples of
167 roughly 2 kg of soil were shaken through a nest of sieves with rectangular holes with an equivalent
168 diameter of 50, 30, and 10 mm and a pan underneath. The aggregate fraction retained on each sieve/pan
169 was oven-dried at 105°C and expressed as a percentage of total dry soil mass. At the time of the analysis,
170 soil water content, measured gravimetrically after the above cited drying was 32% (g g^{-1}), which was
171 considered almost optimal for both soil tillage and for the evaluation of their roughness (Keller et al., 2007).
172 Results were expressed as percentage aggregate size distribution (Van Bavel, 1950). In addition the analysis
173 of the water-stable aggregates before the experiments, obtained using a method already adopted Siegrist

174 *et al.* (1998), highlighted a high level of soil structure (82.4%) confirming the physical (loam texture) and
175 chemical (organic matter) soil fertility.

176

177 *Seed bank analysis*

178 Sampling was performed twice in 2015, before (15 January) and after (2 December) the various agronomic
179 interventions. In each of the 16 experimental plots, 30 soil cores were randomly collected from three
180 different depths (0-10, 10-20 and 20-30 cm) for each of the four replications, for a total of 960 soil samples
181 (10 sampling points^{-plot} x 4 plots x 4 stale seedbed techniques x 3 soil depths x 2 sampling dates). Soil cores
182 (4 cm in diameter and 10 cm long) were taken by means of a metal probe. During the experimental period
183 in no case weeds were capable to have had the time necessary to mature seeds thus avoiding to generate
184 a new seed bank.

185 Seeds were extracted by pre-treating the soil cores for approximately 10 hours in 5 g⁻¹ of sodium
186 hexametaphosphate solution. This allows the dispersal of the soil colloid matrix, thus facilitating the
187 subsequent washing phases. Washing was carried out using a pressure adjustable hydrojet (20-120 bar) to
188 regulate the force of the spray, thereby preventing damage to the seeds (Benvenuti and Pardossi, 2017).
189 Soil samples were washed inside metal cylinders (5 cm diameter and 50 cm long) closed on one side by a
190 removable stopper with a fine metallic mesh (250 µm). The extracted material (seeds, sand, plant residues,
191 etc.) was separated manually by means of a back-lighted magnifying glass (8×). Seeds were then identified
192 with the aid of an optical microscope (45×) and with the aid of special manuals (Montégut, 1971; Davis,
193 1993)

194

195 *Weed seedling emergence evaluation*

196 About 40 days after each of the four soil tillage operations, on the same day as the next tillage, seedling
197 emergence was monitored. Weed seedlings were identified within metal frames (30 cm × 30 cm) placed at
198 the center of the sites (120 sampling points) previously selected for soil extraction. In the control plots

199 where tillage was not performed, seedlings were identified and manually eradicated. This seedling
200 elimination meant that in the following counts, only seedlings that had emerged between two successive
201 soil tillages were considered. The emergence evaluation of each experimental soil tillage type, was carried
202 out on the same days: 20 April, 2 June, 24 July, 8 September and 22 October. In each experimental plot,
203 four sub-plots (0.5-meter squares on each side) were delimited using sticks. In these areas the soil was left
204 undisturbed (no soil tillage was carried out), with manual elimination of the emerged seedlings (on the
205 above-mentioned days of emergence evaluation), in order to quantify the emergence rate in no-till
206 conditions (experimental control).

207 The cumulative emergence data were compared with those of the previous seed bank detected in the
208 same areas. Emergence rate data were expressed as a percentage of the emerged seedlings compared to
209 the pre-existing seed bank: both as a total (layer 0-30 cm) and shallowest (0-10 cm) seed bank.

211 *Weed seed weight measurement*

212 During the years preceding the beginning of the experiments the seeds of the weed populations present in
213 the selected experimental area were collected directly from the senescent mother plants (twenty plants
214 chosen at random for each weed species). Seed weight of each species was determined by weighing 1,000
215 seeds (at the standard storage humidity of about 12%), chosen randomly, according to the International
216 Seed Testing Association rules for seed testing (ISTA, 1999).

218 *Calculation of biodiversity of emerged plant community*

219 The data on the total weed seedling emergence, during the experimental period, were used to calculate
220 the biodiversity and dominance of emerged seedlings according to formulas already widely used in
221 phytosociological studies (Benvenuti and Bretzel, 2017). Shannon diversity index (H') was used to quantify
222 the number of contributing species (species richness) in order to quantify the distribution of individuals

223 between species, and Simpson's index of dominance (D) to measure the probability that two individuals
224 randomly selected from a sample will belong to the same species.

226 *Statistical analysis*

227 All the experiments exploited a randomized complete block design and were conducted with four
228 replicates with a total of 16 plots (4 different soil tillages x 4 replicates). After the normality and
229 homogeneity variance tests, using the Kolmogorov-Smirnov D test and the Cochran test, respectively (Steel
230 and Torrie 1980), the seed bank data and biodiversity indexes were subjected to one-way ANOVA (soil
231 tillage as factor) using the Student–Newman–Keuls test ($p < 0.05$) for mean separation (least-significant
232 difference, LSD). Arcsine transformation was carried out before ANOVA only in the case of data expressed
233 as a percentage (i.e. seed bank distribution, as % of the total, in the several soil layers: 0-10, 10-20 and 20-
234 30 cm). The emergence rate of each tested species and their relative 1,000 seed weight were fitted by the
235 corresponding polynomial regression which described the biological relation between weed seedling
236 emergence and seed weight. For each statistical analysis, CoHort software (1995) was used.

238 **Results**

239 *Seed bank dynamics*

240 Table 1 shows the botanical composition of the seed bank, quantified before the experiments. Over
241 108,000 seeds m^{-2} were detected, confirming the difficulty of weed management in organic cropping
242 systems. Most of the weed species, about 85% had an annual cycle (therophytes), while a small proportion
243 had a perennial cycle (hemicryptophytes and geophytes). An extraordinary abundance of *Sinapis arvensis*
244 (about 42,000 seeds m^{-2}) were found, which alone accounted for about 40% of the whole seed bank. The
245 other five species detected had a least 4,000 seeds m^{-2} : *Portulaca oleracea* (15,650 seeds m^{-2}), *Echinochloa*
246 *crus-galli* (12,390 seeds m^{-2}), *Amaranthus retroflexus* (8,525 seeds m^{-2}), *Lolium multiflorum* (7,640 seeds m^{-2})
247 and *Chenopodium album* (4,330 seeds m^{-2}) with the following percentages (compared to the total): 14.4,

248 11.4, 7.8, 7.0 and 4.0%, respectively. *P. oleracea*, *E. crus-galli* and *A. retroflexus* have high thermal
249 requirements since they are characterized by a C₄ photosynthetic pathway. A total of 49 species, belonging
250 to 23 different botanical families, were identified.

251 The soil aggregate size after the three different stale seedbed techniques (Figure 3) highlights that each
252 tillage had a different degree of soil refinement. The rotary cultivator led to a strong crumbly soil since as
253 much as 70% had aggregate sizes of less than 1%. The spike tooth harrow led to a lesser degree of
254 crumbling keeping about 40% of the clods with dimensions of between 3 and 5 cm and even roughly 15%
255 over 5 cm. The rotary harrow led to an intermediate degree of crumbling about 70% of soil aggregate was
256 between 1 and 3 cm.

257 The reduction of the aforementioned seed bank after the different stale seedbed strategies is shown in
258 Table 2. Soil tillage using the rotary cultivator was the most effective, with a reduction of over 10%. Some
259 weeds were found over 20% such as *Stellaria media*, *Setaria viridis*, *P. oleracea*, *E. crus galli*. In *C. album*, *A.*
260 *retroflexus* and *S. arvensis*, it was even over 30% (33.3, 35.9 and 38.5%, respectively). The rotary harrow
261 was less effective, with a reduction of over 20% in the aforementioned weeds. This soil tillage sequence
262 reduced three weeds by over 25%: *S. arvensis*, *C. album* and *A. retroflexus* (25.7, 26.5 and 27.0%,
263 respectively). In addition to these, another twenty-three species were reduced by over 10%.

264 Soil tillage using the spike tooth harrow showed an almost always significant ($p < 0.05$) less effective
265 reduction than the other soil tillage methods. Despite this, seven weeds were reduced by over 10%
266 (*Alopecurus myosuroides*, *Cynodon dactylon*, *L. multiflorum*, *Poa annua*, *Raphanus raphanistrum*, *S. viridis*
267 and *S. media*) and three others over 15% (*C. album*, *Solanum nigrum* and *S. arvensis*).

268 Finally, the no-till control showed a significantly lower decrease in the final seed bank compared to the
269 initial one. Most species showed less than a 5% decrease and only three poaceae weeds reached a
270 reduction of 10% (*C. dactylon*, *L. multiflorum* and *P. annua*). This trend in tillage efficacy (decreasing from
271 rotary cultivator, rotary harrow, spike tooth harrow and untilled control) was true for nearly all the sampled
272 weeds. However, *P. oleracea* showed that it is a particularly sensitive species to the favourable effect of

273 the crumbling showing a very limited reduction after the spike tooth harrow sequence (only 5.3% and
274 therefore almost unchanged), while this reduction was greater with the rotary harrow (15.4%), and was
275 decidedly higher with the rotary cultivator (35.9%). On the other hand, although *L. multiflorum* was also
276 stimulated to germinate after the soil tillage, it was less dependent on the level of crumbling since the
277 differences between the three types of tillage were decidedly smaller. A similar trend was shown by other
278 poaceae such as *S. viridis*, *P. annua*, *Poa trivialis* *D. sanguinalis*, *C. dactylon* and *A. myosuroides*, since they
279 were less affected by the soil tillage modalities. Two other poaceae, *E. crus-galli* and *Avena sterilis* were an
280 exception since their seed bank depletion was similar to all the other broadleaved species.

281 Before the soil management sequence, the previous seed bank had accumulated over the shallowest soil
282 layers (Figure 4) and decreased with the increasing soil depth. However, after the different tillage
283 sequences, the shallowest (0-10 cm) soil horizon was found the only seed-depleted layer compared to the
284 previous seed bank (Figure 5). This seed decrease in the shallowest soil layer (0-10 cm) was directly related
285 to the type of soil management. The smallest seed quantity (about 15%) was found in the shallowest soil
286 layer (0-10 cm) after the rotary cultivator, while the largest quantity of residual seeds (ungerminated in
287 spite of the soil tillage) was detected after the spike tooth harrow (roughly 32%). An intermediate seed
288 quantity was detected after the rotary harrow (roughly 20%). A cross-comparison between these three
289 shallowest soil layers (after the rotary cultivator, spike tooth harrow or rotary harrow), after subjecting
290 them to the analysis of variance, showed significant (for $p < 0.05$) differences between all of them.

292 *Emergence dynamics*

293 The seedling emergence dynamics of the six most abundant weeds (about 85% of the total seedbank) is
294 shown in Figure 6. *A. retroflexus*, *E. crus-galli* and *P. oleracea* showed the highest emergence rates during
295 the month of May (about 40, 35 and 30% respectively) maintaining a high emergence rate already during
296 the following month of June. On the other hand, *S. arvensis* and *L. multiflorum* showed the highest
297 emergence rates at the beginning (April, roughly 50% in both cases) and at the end (October, roughly 35

298 and 30%, respectively) of the experimental period. *C. album* was in mid-position between these two
299 scenarios. In fact, despite having shown the highest emergence rate at the first sampling carried out in
300 April (roughly 35%), this species maintained a similar emergence in the following month of May (about
301 30%).

302 The emergence rate (Figure 7) was also calculated as the ratio between the previously quantified seed
303 bank (before the tillage sequences) and the emergence dynamics sampled during the experimental period
304 (April-October). The untilled plots showed a very limited (roughly 2%) emergence rate (considering the
305 total 0-30 cm seed bank, Figure 7 A). On the other hand, each type of stale seedbed preparation showed a
306 strong increase in the emergence rate. However, the emergence rate increased by 2% to about 6% after
307 the spike tooth harrow, and to about 10% after rotary harrow. After the rotary cultivator sequence, the
308 emergence rate showed the highest values reaching even 20%. As expected, when the calculation of the
309 emergence rate was related only to the shallowest seed bank (0-10 cm), the rate was much higher (Figure
310 7 B). These emergence rates reached values of about 15% after the spike tooth harrow, 30% after the
311 rotary harrow, and 60% after the rotary cultivator (statistically different values at $p < 0.05$).

312 We then investigated whether or not the germination trigger following the different modalities of stale
313 seedbed preparation was selective towards the various weed species; in other words whether the
314 diversified soil tillage modalities were able to "force" germination uniformly, on all weeds, or whether they
315 elicited germination on certain species.

316

317 *Seed bank biodiversity*

318 The lack of soil tillage sequence led to germination and emergence in only 22 out of 49 species sampled in
319 the seed bank (Figure 8A). However all the stale seedbed preparations increased the number of species
320 although the degree of increase depended on the soil tillage typology. The number of emerged weed
321 species was about 34 and 42 after the spike tooth harrow and rotary harrow sequence, respectively.
322 Similar results were also confirmed by calculation of the dominance Simpson index (D), with maximum

323 values detected in the untilled control (0.22) and the lowest values detected after the rotary cultivator
324 (0.10) (Figure 8 B). Finally, with the Shannon diversity index (H'), the maximum value was found after the
325 rotary cultivator (1.34), while the rotary harrow and spike tooth harrow showed the lowest values of 0.95
326 and 0.73, respectively (Figure 8 C). The untilled control showed the lowest value of 0.51.

327 Finally, Figure 9 shows a significant ($p < 0.05$) polynomial regression between the seed bank emergence rate
328 and 1,000 seed weight of the emerged weeds. As the figure shows, as the weight of 1,000 seeds increased,
329 the seedling emergence rate increased and vice versa.

331 **Discussion**

332 The botanical composition of the seed bank analyzed at the beginning of our experiments (Table 1) is a
333 typical example of long-term organic cropping systems. In fact it was over 100,000 seeds m^{-2} confirming
334 the difficulty of weed management in organic cropping systems, although in a context of high biodiversity
335 as typically occurs in such agroecosystems (Benvenuti and Pardossi, 2017). This weed seed bank was
336 characterized by a high number of species belonging to a high diversification of botanical families. In this
337 “still latent” weed community annual species (therophytes) predominate.

338 From a quantitative point of view this seed bank was larger than those found in other experiments carried
339 out in organic systems of industrial crops (Davis *et al.*, 2005; Riemens *et al.*, 2007; Koocheki *et al.*, 2009).
340 However this quantity was quite similar to those found in organic vegetable crops in other agronomic
341 environments (Benvenuti and Pardossi, 2017) probably due to the poor competitive ability of horticultural
342 crops.

343 The high biodiversity detected in this experiment was, however, in line with those carried out in other
344 agronomic situations (Boguzas *et al.*, 2004; Legere *et al.*, 2005). This substantial seed bank, together with
345 its marked biodiversity, contributes to an ideal experimental agronomic situation. In fact the aim of the
346 experiments was to verify the effectiveness of diversified strategies based on the pre-existing seed bank. A
347 further favourable agronomic situation was that it rained a little even during the hottest periods of full

348 summer (Figure 2). However, the rain did not hinder the planned schedule (approximately on a monthly
349 basis) of the different soil tillage modalities. The degree of soil cloddiness was strongly related to the type
350 of soil tillage (Figure 3), showing a marked crumbling of the aggregate size with the rotary cultivator. These
351 data are in full agreement with previous experiments that have shown that a rotary cultivator, compared
352 to a rotary harrow, seems to produce less cloddiness in the surface layers (Sandri *et al.*, 1998). The
353 literature also confirms the data on the greater roughness shown by the spike tooth harrow (Salem *et al.*,
354 2015). After the seedbed preparation using the spike tooth harrow, the soil roughness was much higher
355 than after the rotary harrow and even more so after the rotary cultivator.

356 However, a further purpose of our research was to relate these data on the physical soil traits to those of
357 the biological fate (seed dormancy, germination, seedling emergence, etc.) of the buried weed seeds. Our
358 analysis of the two types of data provided strong evidence that the degree of soil crumbling was
359 proportional to the germination trigger and to the consequent seedling emergence (Table 2). In fact,
360 considering the total quantified seed bank (layer 0-30 cm), the rotary cultivator sequence, which showed
361 the strongest crumbling of the soil clods, elicited the most marked seed germination "forcing". The
362 consequent seedling emergence reduced the pre-existing seed bank by 20%.

363 The fact that some species responded more intensely to the soil crumbling appears to be due to the
364 respective need for oxygen availability within the micro-environment surrounding the buried seeds. *P.*
365 *oleracea* was found to be particularly stimulated by the degree of soil crumbling but was strongly inhibited
366 by soil burial (Benvenuti *et al.*, 2001) due to its inability to germinate when soil gaseous diffusion
367 (especially in terms of oxygen) is very poor. This oxygen deficiency induces dormancy (Benvenuti and
368 Mazzoncini, 2019), and consequently the soil matrix in the compact clods supports the aging of the seeds.
369 Consequently soil cloudiness acts on both: i) germination inhibition, and ii) seed longevity due to the burial
370 environment (Reus *et al.*, 2001).

371 Other experiments have shown that the seeds of *P. oleracea* have a higher germination after "zero tillage"
372 than after "minimum tillage" (Chauhan and Johnson, 2009). After long-term "zero tillage" management,

373 most seeds likely concentrate in the upper topsoil due to the extremely low self-burial capacity, and
374 consequently they escape by a depth-mediated burial inhibition.

375 Most of the poaceae detected, with the exception of *E. crus-galli* and *A. sterilis*, were only slightly
376 influenced or not at all by soil cloddiness. This could be linked to the typical ecology of grasses that form a
377 transient seed bank (Thompson et al., 1993). These species usually accumulate their seeds on the soil
378 surface and tend to trigger germination in a way that is less dependent on the degree of soil softness. In
379 fact in cropping systems characterized by long-term “zero tillage” (therefore with little softness), weeds
380 belonging to the poaceae botanic family tend to be particularly predominant (Webster *et al.*, 2003).

381 It is not clear which soil layers, after seed bed preparation, were affected by germination and the
382 consequent seed bank reduction. The architecture of the vertical seed arrangement thus needs to be
383 investigated after the various seedbed preparation strategies have been implemented. Each type of soil
384 tillage, although to different extents, reduced the seed bank almost exclusively in the shallowest soil layer
385 (0-10 cm). This confirms that the seed burial depth plays a crucial role in germination-inhibition and
386 consequently maintains most of the seed bank. In fact the soil physics showed a strong influence on the
387 dormancy/germination performance since a poor gaseous diffusion (as occurs inside compacted clods)
388 appears more suitable for accumulating a substantial seed bank. In these seedbed preparations, a rotary
389 cultivator (Figure 5), seems to be the most effective in hindering dormancy and consequently the long-
390 term storage of seeds in the soil. In fact, in our experiments, the shallowest soil layer (0-10 cm) showed a
391 strong seed depletion, and constituted only about 10% of the residual seed bank. This ability to “force”
392 germination appears to be linked to the high degree of soil crumbling (see Figure 3) which increases soil
393 gas diffusion and consequently triggers buried seed germination. The hypothesis of a direct relationship
394 between soil crumbling, gaseous diffusion and germination trigger was confirmed by the lower seed bank
395 depletion within the same soil layer (0-10 cm) after rotary harrowing and, even less, after the spike tooth
396 harrow. This does not necessarily mean that the most agronomically appropriate method is to use a rotary
397 cultivator. It is important to remember that soil crumbling also elicits oxidation of the soil organic matter

398 (Balesdent et al., 2000). Unfortunately mechanical weed control methods are not compatible with
399 protecting the organic matter in the soil.

400 Unfortunately the seed bank of the underlying soil layers (10-20 and 20-30 cm) was not affected by any of
401 the types of tillage. This appears due not only to the tillage depth (15 cm) but also to the typical
402 germination inhibition due to burial depth (Benvenuti and Mazzoncini, 2019). It should be noted that
403 although the botanical structure of seed bank also include perennial species, their scarce quantity has
404 made negligible the emergence rate deriving from vegetative organs.

405 Clearly the emergence dynamics, triggered by soil tillage, were influenced by the ecological needs (above
406 all in terms of temperature) of each weed species tested (Figure 6). Consequently if the aim of stale
407 seedbed preparation is to reduce the seed bank of certain predominant weed species (spring-summer or
408 autumn-winter cycle), soil tillage needs to be carried out during the most suitable periods (early or late
409 spring). For example, *A. retroflexus* and *E. crus-galli* showed the most intense periods of emergence at the
410 beginning of June confirming the rather high base temperatures (about 12°C) for germination (Masin et al.,
411 2010). Similarly, but occurring earlier, the emergence dynamics of *C. album* showed lower thermal
412 requirements than *A. retroflexus* and *E. crus-galli* (Leblanc et al., 2004). On the other hand *P. oleracea* had
413 a greater, well known (Baskin and Baskin, 1988), thermal requirement, since their emergence peak occurs
414 during June and also partially in full summer. The overlap of these data on the thermal requirements of *P.*
415 *oleracea* with the need for soil crumbling highlights that the most appropriate preventive method to
416 control this species consists in a seedbed preparation using the rotary cultivator in full summer.

417 On the other hand, the remaining prevalent species, such as *S. arvensis* and *L. multiflorum*, were sensitive
418 to the soil tillage especially during the earliest periods (April). In these cases, the overlap of their period of
419 emergence with the respective soil crumbling needs (higher for *S. arvensis* and lower for *L. multiflorum*)
420 highlighted the following optimal preventive control methods: early seedbed preparation in both cases but
421 using the rotary cultivator for the predominance of *S. arvensis* and using whatever tillage for *L. multiflorum*.

422 In fact *L. multiflorum* showed an appreciable emergence rate even after the spike tooth harrow, in spite of
423 their lower activity in the crumbling soil clods.

424 In terms of the effectiveness of the seedbed preparation period, our results may appear to be
425 disappointing since even in the best case of the rotary cultivator (Figure 7A), only about 20% of the total
426 seed bank (0-30 cm) was induced to germinate. This thus provides evidence that the buried seeds had very
427 little stimulus to trigger germination without any mechanical soil disturbance confirming similar recent
428 studies (Torra *et al.*, 2018). However if we only consider the surface layer, the seed bank reduction was
429 much greater, not only with the rotary cultivator but also with rotary harrowing and to a lesser extent with
430 spike tooth harrowing. This drastic reduction in the shallowest seed bank is of notable agronomic
431 importance in preventing the weed invasion of the next crops since the "active seed bank" (0-10 cm) was
432 strongly depleted. This thus confirmed that the seedbank is active above all, or perhaps exclusively, when
433 the seed burial depth is less than 10 cm. It should be noted that although suicidal germinations are
434 possible (germination not followed by emergence) which could underestimate the seed bank depletion,
435 this was found a rare event (Benvenuti *et al.*, 2001) and consequently it is considered negligible.

436 Another important result is that each seedbed preparation depleted the seed bank in a non-selective way.
437 In fact in all the stale seedbed strategies, the emerged weed communities showed a higher biodiversity,
438 and a lower dominance, with respect to the no-till control (Figure 8). This was particularly true after the
439 use of the rotary cultivator. The greater soil crumbling probably triggered germination even in those
440 species that are particularly affected by inhibition due to the limiting gas diffusion in the soil clods. In fact
441 the lack of oxygen around the buried seeds, incorporated into the micro-clods, induced dormancy (Benech-
442 Arnold *et al.*, 2000).

443 It is still not clear whether there is a correlation between this germination-inhibition due to the soil clods
444 and the biodiversity reduction of the emerged species. A possible correlation was suggested by the
445 following observation: several of the weed species that were not present, or present in low quantities, as
446 emerged flora in the case of a minor soil crumbling (i.e. spike tooth harrowing) and even more so in the

447 case of the no-till control, had small sized seeds. This suggests that additional data (1,000 seed weight)
448 should be analysed in order to verify whether the size of seeds plays a key role or not. A significant
449 polynomial regression ($p < 0.05$) confirmed that small seeds showed a higher soil inhibition since their
450 emergence rate was proportional to the 1,000 seed weight.

451 The weed species characterized by small seeds are thus strongly inhibited by soil burial thus allowing their
452 long-term persistence. In practice, the depth of burial of weed species characterized by small seeds acts as
453 a filter that hinders germination already over a few millimetres of burial despite the softening of the soil by
454 tillage. These results are in full agreement with Gardarin et al. (2010) who found a close relationship
455 between weed seed traits and the physical environment of the soil. The stale seedbed preparation thus
456 appears be less effective against species with small seeds which therefore tend to form a persistent seed
457 bank. Basically, smaller seeds are less stimulated to germinate by the soil softening induced by the tillage,
458 thus revealing a marked soil-mediated germination inhibition (Torra et al., 2018).

459 This hypothesis is also supported by the evidence that in no-tillage systems, most small seeds promote
460 secondary dormancy (Ghersa and Martinez-Ghersa, 2000) thus allowing a longer-living seed bank.

462 **Conclusions**

463 Our experiments clearly showed that the degree of soil crumbling was strongly related to the triggering of
464 the seed bank germination and consequently to the effectiveness of the seedbed preparation. The
465 achievement of about 60% of the emergence rate of the shallowest seed bank (0-10 cm), using the rotary
466 cultivator, is an extremely encouraging result. In addition the deeper soil crumbling was able to even
467 stimulate the germination of small seeds despite their marked tendency to enter dormancy within the soil
468 clods. It is thus crucial to improve knowledge of the seedbed preparation strategies available in terms of
469 the dynamics of both agronomic parameters: seed bank and organic matter. This should lead to the
470 optimal compromise between agronomic positivity and negativity (seed bank depletion and organic matter

471 oxidation respectively) in relation to the choice of the stale seedbed strategy in terms of both: i) typology
472 (rotary cultivator, rotary harrowing, spike tooth harrowing, or others) and ii) frequency.

473 The best tillage time (early or late) needs to be ascertained in order to maximize their germination in
474 relation to the thermal requirements of the prevalent weed species.

475 Irrespectively of the kind of stale seedbed preparation, any soil layer inversion (i.e. plowing) should not
476 take place before the subsequent crop planting, so as not to bring the deeper unchanged seed bank
477 towards the soil surface (Mohler et al., 2006) thus allowing a reduction of emergence dynamics due to the
478 weed seed depletion of the upper topsoil where typically occurs almost all germinations (Benvenuti et al.,
479 2001). Weed seedling emergence will thus be decidedly lower and consequently it will be possible to
480 defend the next crop with the curative means in a sustainable way (Chauhan et al., 2012).

481 In summary, the stale seedbed technique studied appears be useful for all cropping systems but appears to
482 be of crucial importance in the case of organic cropping systems since their agronomic sustainability will be
483 increasingly dependent on the preventive tools used for weed management of the agroecosystem.

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667

668 **Table 1.** Botanical information and density (absolute and relative) weed seedbank (0-30 cm) sampled before the experiments.

Species	Botanic family	Weed type ¹	1,000 seed weight (g)	Life form ²	Photosynthetic pathway	Seed bank	
						Absolute density (seeds m ⁻²)	Relative density ³ (%)
<i>Abutilon theophrasti</i> L.	Malvaceae	B	9.23	T	C ₃	430	0.40
<i>Alopecurus myosuroides</i> Hudson.	Poaceae	G	1.98	T	C ₃	235	0.22
<i>Amaranthus retroflexus</i>	Amaranthaceae	B	0.42	T	C ₄	8,525	7.88
<i>Anagallis arvensis</i> L.	Primulaceae	B	0.51	T	C ₃	755	0.70
<i>Avena sterilis</i> L.	Poaceae	G	31.2	T	C ₃	65	0.06
<i>Bromus sterilis</i> L.	Poaceae	G	9.42	T	C ₃	65	0.06
<i>Capsella bursa-pastoris</i> L.Med.	Brassicaceae	B	0.08	T	C ₃	80	0.07
<i>Cerastium glomeratum</i> Thuill.	Caryophyllaceae	B	0.05	T	C ₃	25	0.02
<i>Chenopodium album</i> L.	Chenopodiaceae	B	0.46	T	C ₃	4,330	4.00
<i>Cirsium arvense</i> L.Scop.	Asteraceae	B	1.34	G	C ₃	450	0.42
<i>Convolvulus arvensis</i> L.	Convolvulaceae	B	14.5	G	C ₃	65	0.06
<i>Conyza canadensis</i> (L.) Cronq.	Asteraceae	B	0.07	T	C ₃	55	0.05
<i>Cynodon dactylon</i> (L.) Pers.	Poaceae	G	0.31	G	C ₄	140	0.13
<i>Daucus carota</i> L.Scop.	Apiaceae	B	1.12	H	C ₃	55	0.05
<i>Digitaria sanguinalis</i> (L.) Scop.	Poaceae	G	0.51	T	C ₄	235	0.22
<i>Echinochloa crus-galli</i> L.Beauv.	Poaceae	G	0.87	T	C ₄	12,340	11.40
<i>Euphorbia helioscopia</i> L.	Euphorbiaceae	B	2.28	T	C ₃	135	0.12
<i>Fumaria officinalis</i> L.	Papaveraceae	B	3.12	T	C ₃	345	0.32
<i>Galium aparine</i> L.	Rubiaceae	B	8.81	T	C ₃	45	0.04
<i>Geranium dissectum</i> L.	Geraniaceae	B	2.25	T	C ₃	75	0.07
<i>Heliotropium europaeum</i> L.	Boraginaceae	B	1.13	T	C ₃	35	0.03
<i>Lactuca serriola</i> L.	Asteraceae	B	0.57	T	C ₃	15	0.01
<i>Lamium amplexicaule</i> L.	Lamiaceae	B	0.61	T	C ₃	35	0.03
<i>Lamium purpureum</i> L.	Lamiaceae	B	0.95	T	C ₃	125	0.12
<i>Lolium multiflorum</i> Lam.	Poaceae	G	2.94	T	C ₃	7,640	7.06
<i>Malva officinalis</i> L.	Malvaceae	B	5.52	H	C ₃	35	0.03
<i>Matricaria chamomilla</i> L.	Asteraceae	B	0.09	T	C ₃	320	0.30
<i>Mercurialis annua</i> L.	Euphorbiaceae	B	2.03	T	C ₃	75	0.07
<i>Papaver rhoeas</i> L.	Papaveraceae	B	0.14	T	C ₃	950	0.88
<i>Picris echioides</i> L.	Asteraceae	B	1.22	T	C ₃	155	0.14
<i>Picris hieracioides</i> L.	Asteraceae	B	0.96	H	C ₃	120	0.11
<i>Plantago lanceolata</i> L.	Plantaginaceae	B	1.42	H	C ₃	85	0.08
<i>Poa annua</i> L.	Poaceae	G	0.28	T	C ₃	2,330	2.15
<i>Poa trivialis</i> L.	Poaceae	G	0.12	T	C ₃	1,450	1.34
<i>Polygonum aviculare</i> L.	Polygonaceae	B	1.29	T	C ₃	1,650	1.52
<i>Polygonum convolvulus</i> L.	Polygonaceae	B	1.48	T	C ₃	35	0.03
<i>Polygonum persicaria</i> L.	Polygonaceae	B	2.04	T	C ₃	1,850	1.71
<i>Portulaca oleracea</i> L.	Portulacaceae	B	0.11	T	C ₄	15,650	14.46
<i>Ranunculus arvensis</i> L.	Ranunculaceae	B	10.2	T	C ₃	650	0.60
<i>Raphanus raphanistrum</i> L.	Brassicaceae	B	11.45	T	C ₃	75	0.07
<i>Rumex crispus</i> L.	Polygonaceae	B	3.32	H	C ₃	355	0.33
<i>Senecio vulgaris</i> L.	Asteraceae	B	0.24	T	C ₃	465	0.43
<i>Setaria viridis</i> L.Beauv.	Poaceae	G	2.27	T	C ₃	1,120	1.03
<i>Sinapis arvensis</i> L.	Brassicaceae	B	1.82	T	C ₃	42,450	39.22
<i>Solanum nigrum</i> L.	Solanaceae	B	0.79	T	C ₃	45	0.04
<i>Sonchus oleraceus</i>	Asteraceae	B	0.34	H	C ₃	95	0.09
<i>Stellaria media</i> L.Vill.	Caryophyllaceae	B	0.38	T	C ₃	385	0.36
<i>Verbena officinalis</i> L.	Verbenaceae	B	0.35	H	C ₃	255	0.24
<i>Veronica persica</i> Poiret	Scrophulariaceae	B	1.04	T	C ₃	1,335	1.23
Total seed bank						108,235	100

669 1 B= broadleaf; G= grasses

670 2 T=Therophyte; G= Geophyte; H= Hemicriptophyte

671 3 = density percentage of each species to respect to the total.

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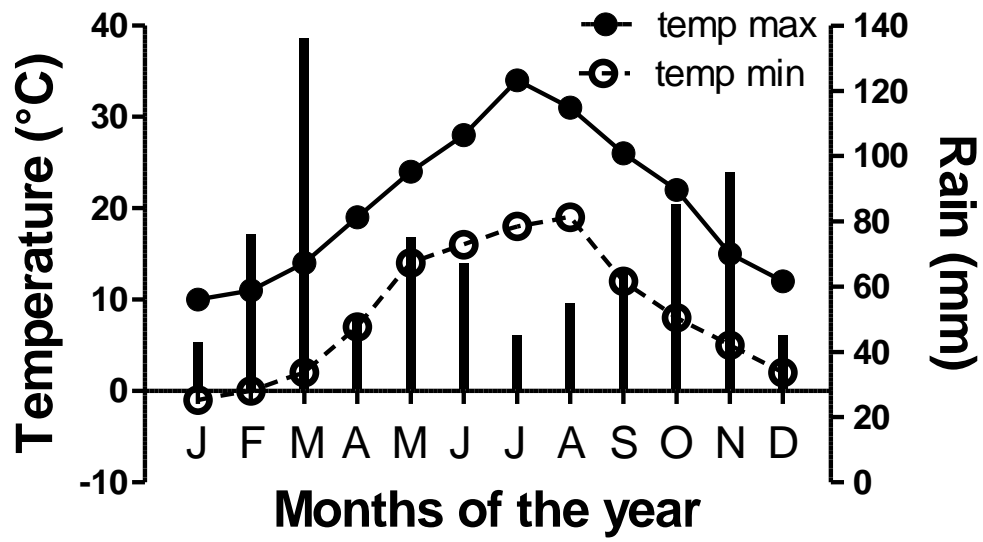
Table 2. Amount of seed bank reduction of the several weed species (difference % between the initial and final seed bank within the total soil layer 0-30 cm) and the residual total seed bank (at the end of experiments) as absolute density (seeds m⁻²) after the different stale seedbed techniques. Means followed by different letter, within each line, show statistical difference to ANOVA (p< 0.05).

Weed species	Seed bank reduction after different stale seedbed techniques (%)			
	Rotary cultivator	Spike tooth harrow	Rotary harrow	Control (untilled)
<i>Abutilon theophrasti</i>	15.58 a	9.77 b	10.23 b	1.53 c
<i>Alopecurus myosuroides</i>	14.04 a	10.55 c	13.38 a	3.23 d
<i>Amaranthus retroflexus</i>	38.52a	9.95 c	27.06 b	4.32 d
<i>Anagallis arvensis</i>	14.90 a	3.91 b	3.78 b	2.45 c
<i>Avena sterilis</i>	20.00 a	6.15 c	12.31 b	3.21 d
<i>Bromus sterilis</i>	15.38 a	3.08 c	13.85 b	2.28 c
<i>Capsella bursa-pastoris</i>	15.05 a	3.75 b	14.25 a	1.18 c
<i>Cerastium glomeratum</i>	14.92 a	3.04 b	3.34 b	2.62 c
<i>Chenopodium album</i>	33.31 a	15.38 c	26.50 b	8.45 d
<i>Cirsium arvense</i>	12.00 a	5.11 c	7.33 b	3.43 d
<i>Convolvulus arvensis</i>	13.85 a	6.77 c	10.77 b	4.02 d
<i>Conyza canadensis</i>	15.45 a	3.55 b	13.64 a	3.24 b
<i>Cynodon dactylon</i>	15.71 a	11.86 b	13.57 a	10.32 b
<i>Daucus carota</i>	10.91 a	5.45 c	8.49 b	3.45 d
<i>Digitaria sanguinalis</i>	15.49 a	9.79 b	14.04 a	8.87 b
<i>Echinochloa crus-galli</i>	26.21 a	6.87 c	10.90 b	4.45 d
<i>Euphorbia helioscopia</i>	11.11 a	3.70 c	5.93 b	2.32 d
<i>Fumaria officinalis</i>	10.14 a	6.12 b	7.83 b	4.56 c
<i>Galium aparine</i>	13.33 a	7.25 b	11.11 a	6.34 b
<i>Geranium dissectum</i>	10.67 a	5.33 b	6.67 b	2.32 c
<i>Heliotropium europaeum</i>	16.29 a	8.57 c	12.57 b	5.57 d
<i>Lactuca serriola</i>	13.33 a	6.67 b	7.12 b	6.85 b
<i>Lamium amplexicaule</i>	11.43 a	3.71 c	5.71 b	3.58 c
<i>Lamium purpureum</i>	18.40 a	5.60 c	8.40 b	4.43 d
<i>Lolium multiflorum</i>	15.04 a	10.45 b	11.62 b	11.97 b
<i>Malva officinalis</i>	11.43 a	6.67 b	5.71 b	3.45 c
<i>Matricharia chamomilla</i>	14.69 a	1.79 c	3.44 b	1.58 c
<i>Mercurialis annua</i>	14.67 a	7.04 c	9.33 b	6.89 c
<i>Papaver rhoeas</i>	11.58 a	1.79 b	9.32 a	1.65 b
<i>Picris echioides</i>	14.84 a	7.74 b	9.68 b	7.45 b
<i>Picris hieracioides</i>	12.50 a	3.33 c	5.83 b	3.12 c
<i>Plantago lanceolata</i>	11.24 a	3.53 c	5.88 b	3.58 c
<i>Poa annua</i>	19.76 a	10.52 b	17.64 a	10.45 b
<i>Poa trivialis</i>	18.50 a	8.48 b	16.90 a	9.23 b
<i>Polygonum aviculare</i>	10.80 a	3.39 c	6.18 b	4.24 c
<i>Polygonum convolvulus</i>	11.43 a	5.71 b	9.57 a	6.25 b
<i>Polygonum persicaria</i>	13.24 a	9.57 b	10.22 b	6.88 c
<i>Portulaca oleracea</i>	26.26 a	5.36 c	15.40 b	2.24 d
<i>Ranunculus arvensis</i>	18.76 a	6.62 b	6.92 b	5.57 b
<i>Raphanus raphanistrum</i>	14.67 a	11.67 b	12.00 b	9.73 c
<i>Rumex crispus</i>	18.68 a	9.23 c	13.86 b	8.34 c
<i>Senecio vulgaris</i>	12.31 a	6.24 b	6.88 b	4.55 c
<i>Setaria viridis</i>	22.95 a	11.25 c	16.92 b	4.23 d
<i>Sinapis arvensis</i>	35.94 a	18.23 c	25.97 b	4.45 d
<i>Solanum nigrum</i>	18.89 a	16.33 c	12.67 b	2.23 d
<i>Sonchus oleraceus</i>	16.32 a	5.26 b	15.26 a	2.45 b
<i>Stellaria media</i>	22.49 a	12.08 c	16.94 b	5.87 d
<i>Verbena officinalis</i>	14.71 a	1.96 c	7.14 b	1.11 c

<i>Veronica persica</i>	18.51 a	6.94 c	10.34 b	2.56 d
Residual seed bank (absolute density seeds m ⁻²)	75,450 a	84,760 c	79,615 b	106,335 d

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Figure 1. Meteorological data (rainfall, maximum and minimum temperature) occurred during the experimental year 2015

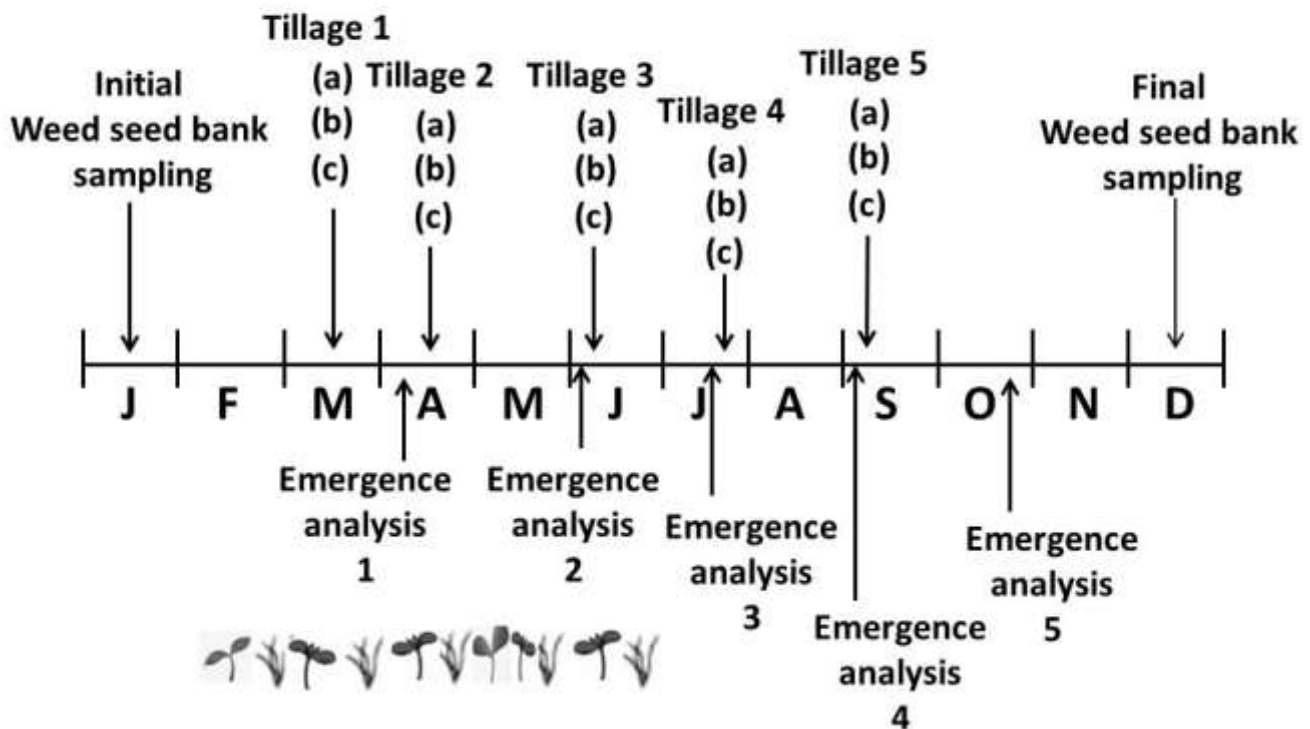


Figure 2. Schematic representation of the soil tillage types and sequence (a= rotary cultivator, b= spike tooth harrow, c= rotary harrow) and times of the experimental evaluations (seedbank and emergence analyses).

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Figure 3. Illustration of the soil tillage methods of the tested “stale seedbed preparation” (A1= rotary cultivator, B1= spike tooth harrow, C1= rotary harrow), the related tools (2A, 2B and 2C) and the visual effect on the respective weed emergence dynamics (detected in July two weeks after of the diversified soil management 3A, 3B and 3C respectively).

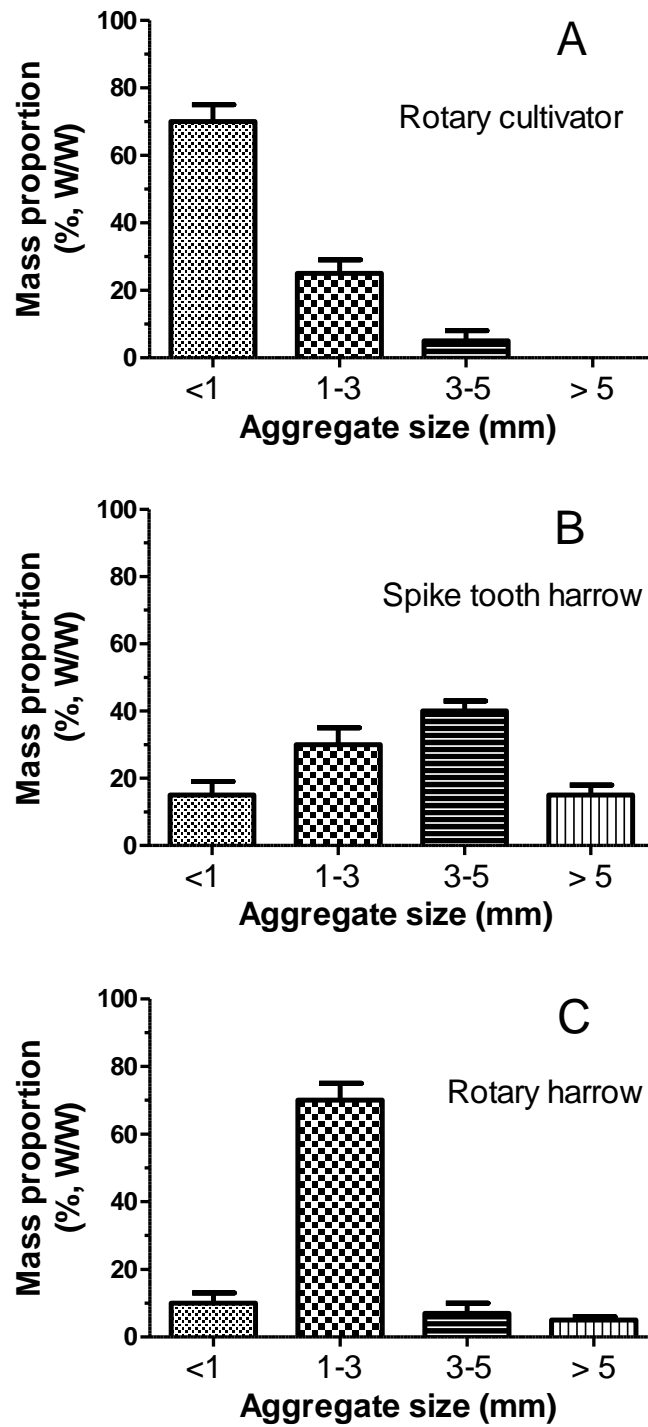
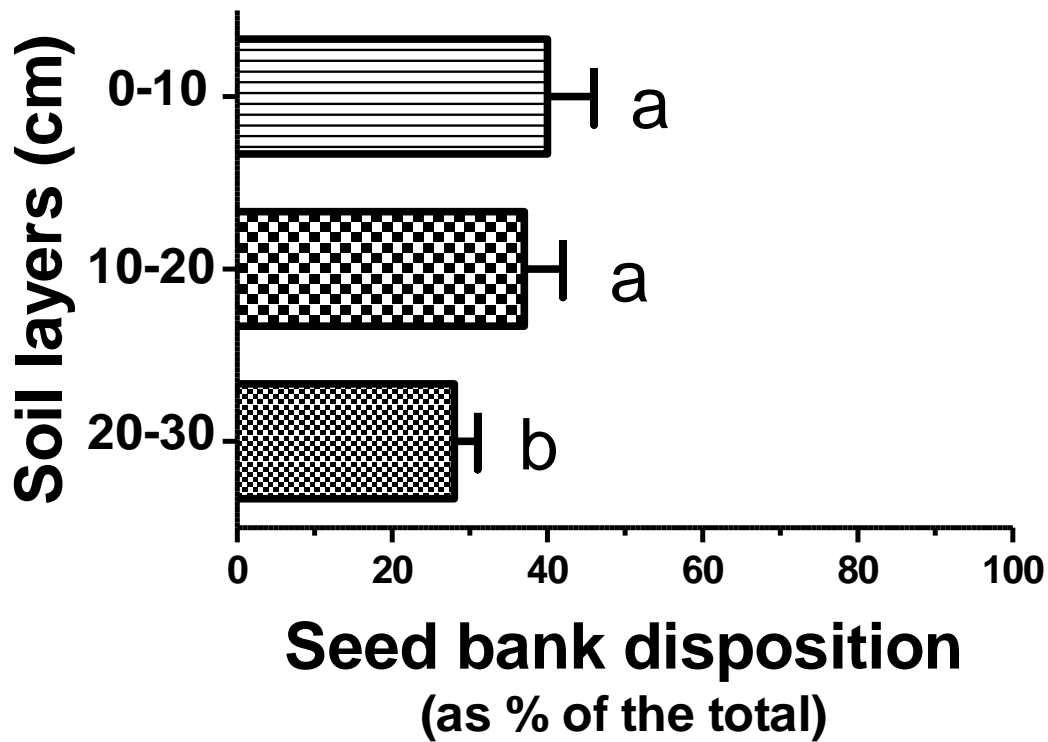


Figure 3. Graphic representation of the dimensional composition of the soil aggregates (mass proportion, % g^{-g}, of the following aggregate size fractions: <1, 1-3, 3-5 and >5 cm) after the diversified tillage. Vertical bars indicate standard errors of the mean.



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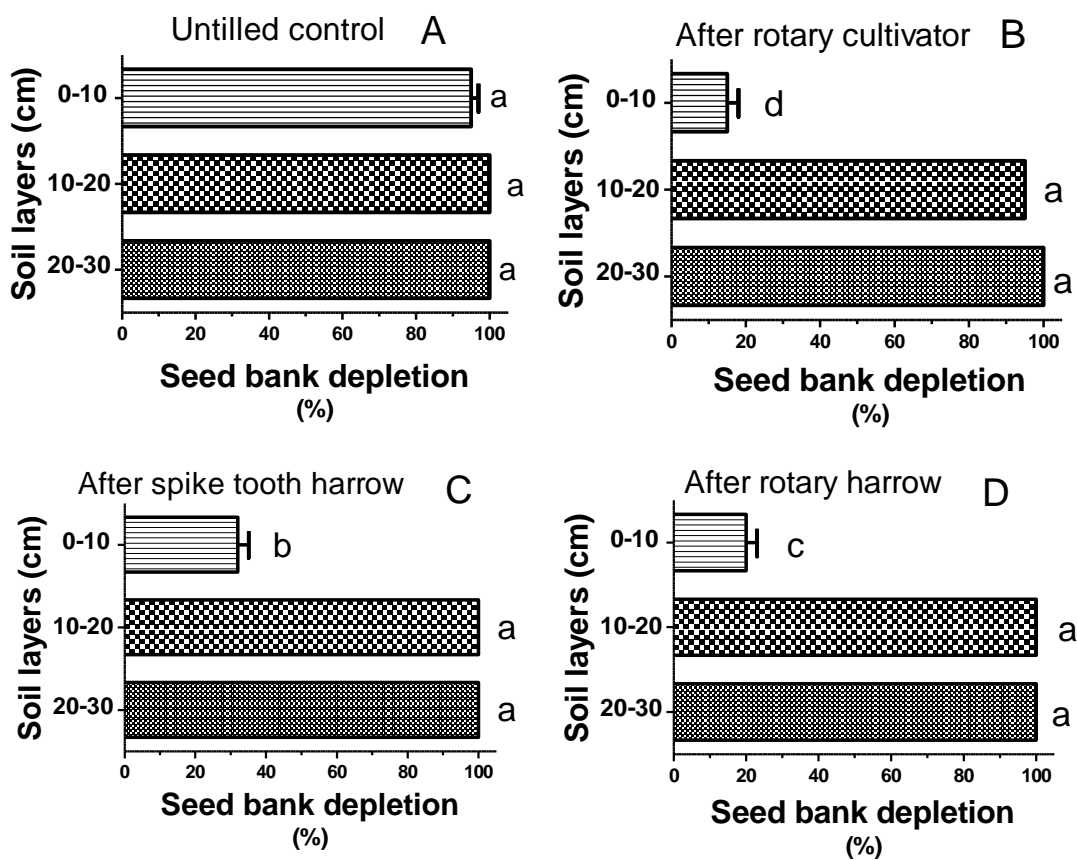
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Figure 4. Seed bank disposition in the several soil layers (0-10, 10-20 and 20-30 cm) before the experimental period. Vertical bars indicate standard errors of the mean. Means followed by different letters show statistical difference for $p < 0.05$ according to the Student–Newman–Keuls test.

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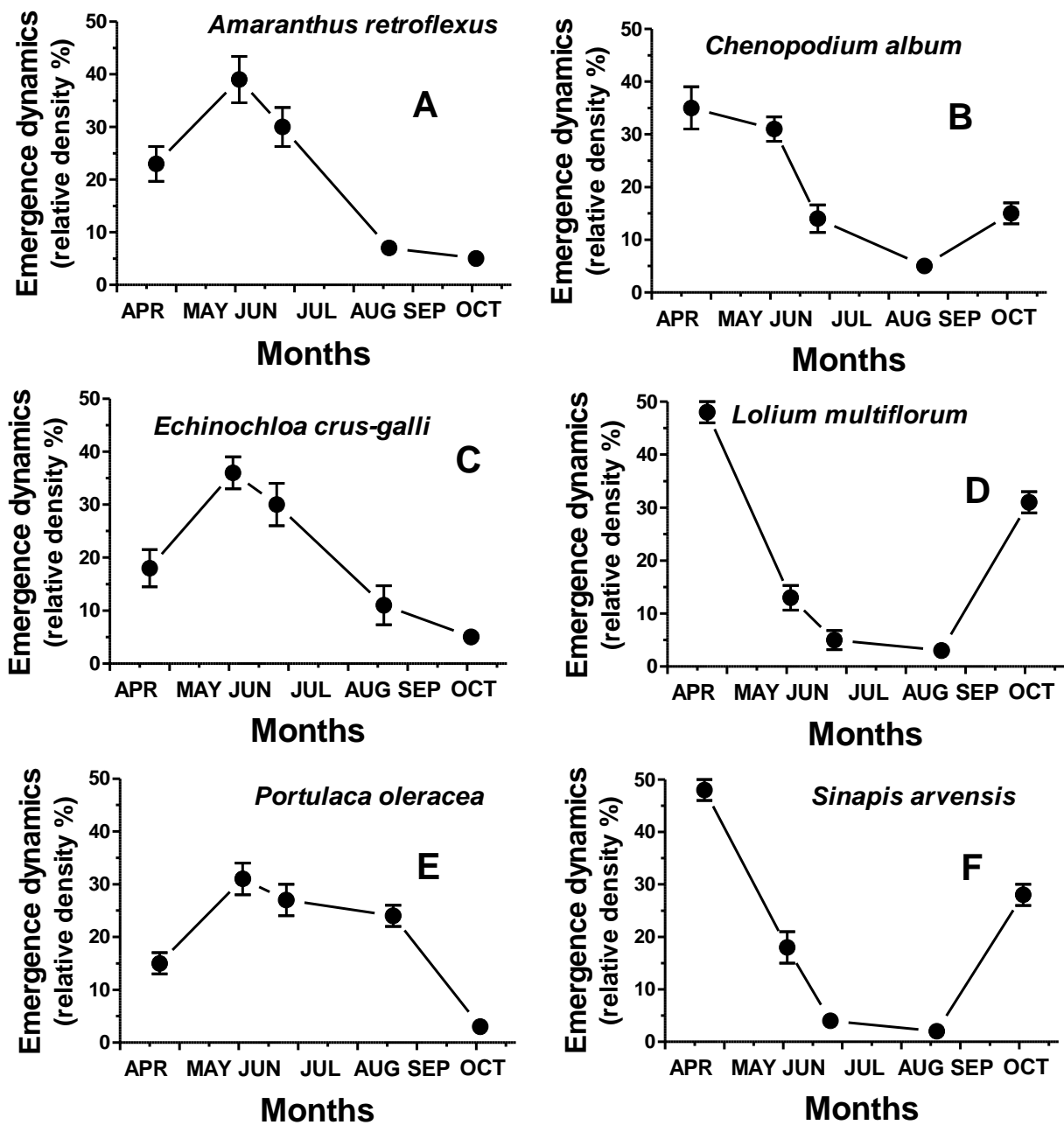
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Figure 5. Seed bank depletion expressed as % of the previous seed bank for each soil layer (0-10, 10-20 and 20-30 cm) after different soil management: untilled control (A), rotary cultivator (B), spike tooth harrow (C) and rotary harrow (D). Vertical bars indicate standard errors of the mean. Means followed by different letters show statistical difference for $p < 0.05$ according to the Student–Newman–Keuls test.

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Figure 6. Emergence dynamics during the several months of experimental period (as % of the cumulative emergence) of the six most abundant weed: *A. retroflexus*, *C. album*, *E. crus-galli*, *L multiflorum*, *P.oleracea* and *S. arvensis*. The data of the different tillage techniques were pooled due to the lack of any interaction. Horizontal bars indicated \pm standard error of the means.

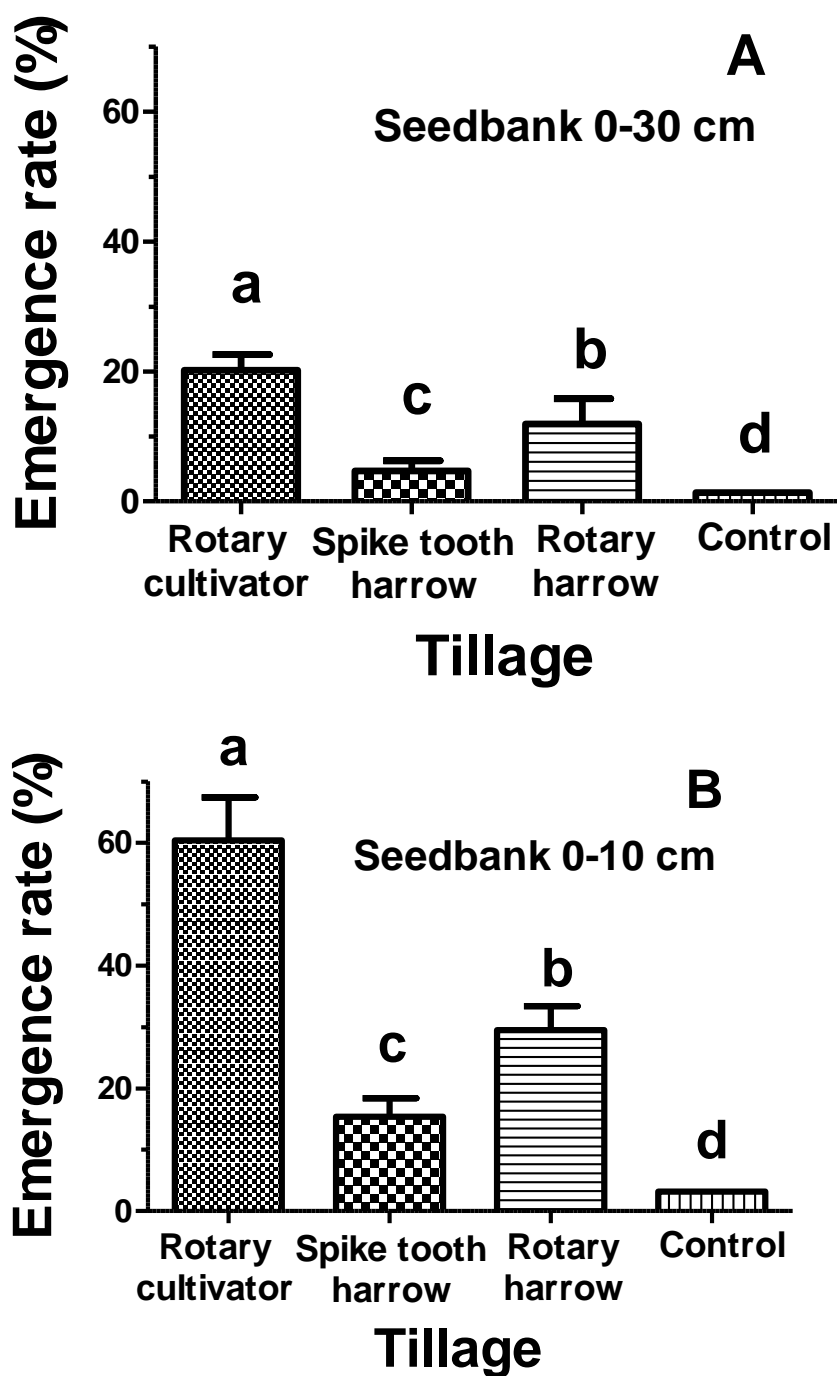
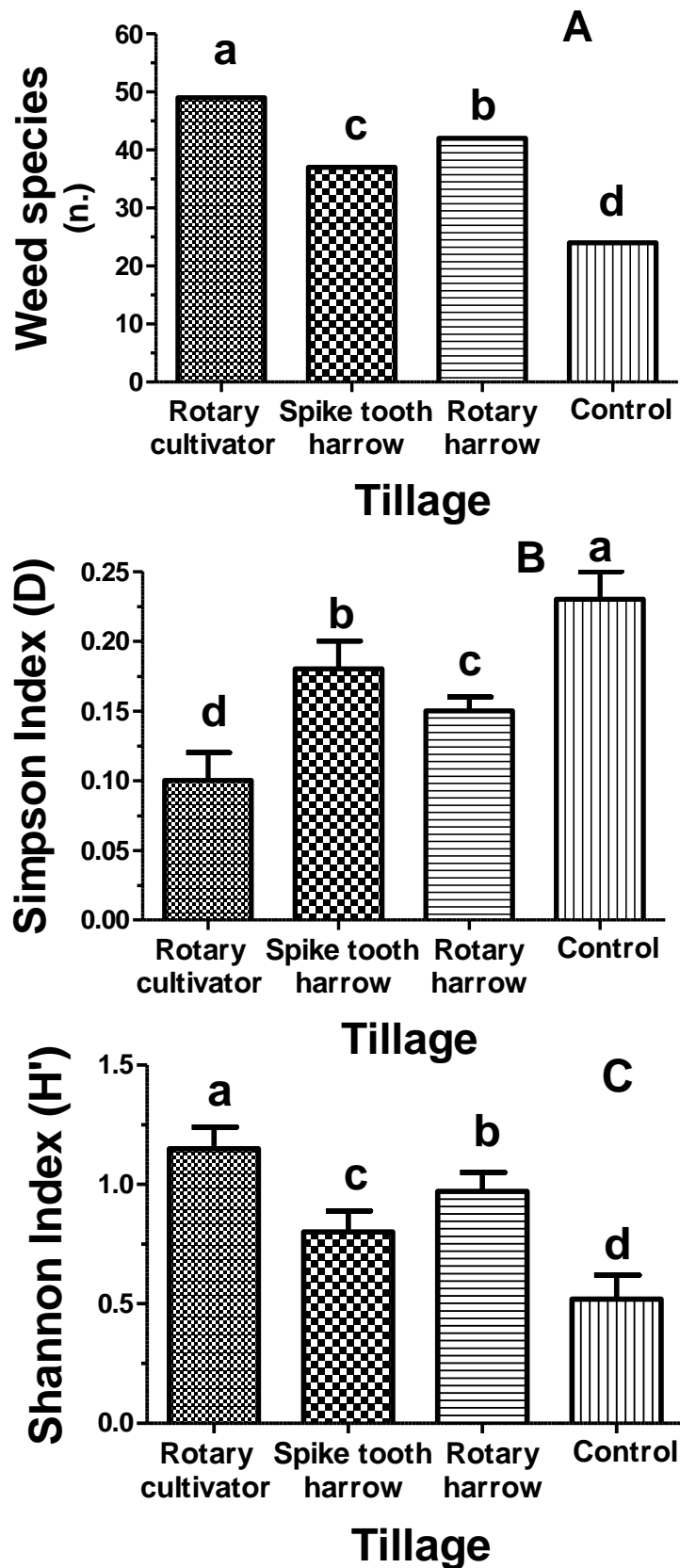


Figure 7. Emergence rate of the different tillage management (rotary cultivator, spike tooth harrow, rotary harrow and undisturbed control) expressed as % referred to the total analyzed seed bank (0-30 cm, A) or referred the only shallowest soil layer (0-10 cm, B). Vertical bars indicate standard errors of the mean. Means followed by different letters show statistical difference for $p < 0.05$ according to the Student–Newman–Keuls test.



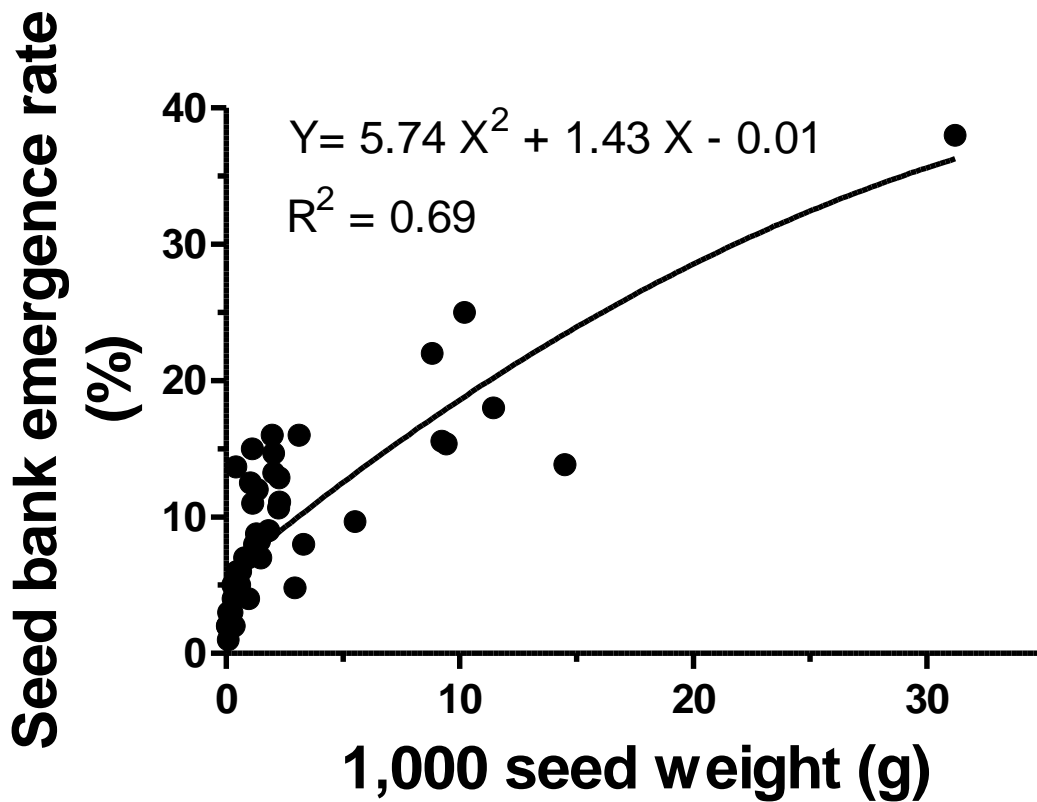
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Figure 8. Indexes of biodiversity (Shannon H' (A) and dominance (Simpson, D , (B) and number of emerged weed species (C) as a function of the various tillage managements: rotary cultivator, spike tooth harrow,

762 rotary harrow and undisturbed control. Vertical bars indicate standard errors of the mean. Means followed
763 by different letters show statistical difference for $p < 0.05$ according to the Student–Newman–Keuls test.
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 766 **Figure 9.** Polynomial regression between seed bank emergence rate (as % of the shallowest soil layer, 0-10
 767 cm) and 1,000 seed weight of the corresponding weed species. The data of the emergence rate are
 768 referred only to the stale seedbed preparation carried out by rotary cultivator since this was the only soil
 769 tillage capable to trigger germination to all of the pre-existing seed bank. The equation (significant for P >
 770 0.05) and the corresponding R² value was shown.