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

Running head: Stale seedbed for weed control

Keywords

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

Highlights

► The degree of soil crumbling measured by clods size is proportional to the weed emergence rate.

► A decrease in the seed bank occurs only within the shallowest soil layer (0-10 cm).

► Greater soil crumbling allows the germination trigger of a higher number of species.

► The smaller and lighter seeds show the lower emergence rate due to their greater burial intolerance.

► Stale seedbed preparation sequences can play a crucial role in the weed management sustainability.

Introduction

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

Unfortunately this agronomic simplification, which evolved in the post-war period during the so-called “green revolution” (Evenson and Gollin, 2003), has made the crop protection more vulnerable by the dominance of more aggressive weed species. Unfortunately the discovery of effective herbicides means that preventive methods have been superseded by curative methods without looking for an integration between them. Yet the use of herbicides alone is unlikely a decisive remedy and is only effective in the long-term when the efficacy shown in a single year can be maintained for a long period thanks to an integrated weed management (Swanton et al., 2008) using a wide range of agronomic practices.

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

The extreme scarcity of effective curative methods in sustainable agricultural systems makes preventive operations even more crucial (Pannacci et al., 2017). In fact, the greatest critical issue in the economic sustainability of organic agriculture is due to the extreme abundance of weed populations, resulting in a substantial drop in crop yields (Seufert et al., 2012). Except for unusual and specific eco-compatible methods (Li et al., 2012), these infestations are difficult to manage in the long term in organic cropping systems due to the heavy "seed rain". This leads to the accumulation of a considerable amounts of seeds in the soil for both arable (Teasdale et al., 2004) and horticultural (Benvenuti and Pardossi, 2017) crops.

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

The agronomic “forcing” of buried weed seed germination is the main agronomic strategy used to deplete the seedbank. Unfortunately this is hindered by the typical physiological (Vleeshouwers et al., 1995), physical (Paulsen et al., 2013) and/or environment-mediated (Benech-Arnold et al., 2000) seed dormancy. The last kind of dormancy is called secondary dormancy (Hilhorst, 1998).

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

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

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

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

Material and methods

Agronomic environment

The experiments were carried out in 2015 in Tuscany near Sansepolcro, (Italy, 43° 36’ North, 10° 20’ East) at the Aboca Farm specialized in the production and processing of medicinal herbs using organic cropping systems. The experimental area (roughly 10 ha) was selected due to its uniformity of management in terms of soil texture and previous agronomic practices. In the last 10 years the following species had been rotated: Chamomile (*Matricaria chamomilla* L.), Purple Coneflower (*Echinacea purpurea* L.), Mallow (*Malva sylvestris* L.), Passionflower (*Passiflora incarnata*), and Dandelion (*Taraxacum officinale* L.). Throughout the 10-year period, the same tillage techniques had been used: ploughing to 25 cm and using disk harrow for seedbed preparation. This area is also characterized by a marked uniformity in terms of both: i) pedologic characteristics (USDA classified xerofluvent loam soil, 65% sand, 20% lime, 15% clay; pH 7.2, 1.8 organic matter); and ii) botanical structure and quantity of existing weed communities. In particular, it should be noted that the previous rotation of medicinal crops (often characterized by multi-year agronomic cycle), had selected weed communities of both: autumn-winter and spring-summer cycle.

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

Previous experimental problems

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

Stale seedbed preparation techniques

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

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

Soil aggregate size evaluation

Soil samples were collected after the tillage intervention of 4 June when the soil moisture conditions were assessed as optimal for this evaluation. This sampling was carried out from a 0-10 cm layer in each plot using a rectangular trough (15 cm x 17.5 cm) with minimal disturbance and samples were sealed in plastic bags according to Kemper and Rosenau (1986). The soil was exposed to air dry for three days. Samples of roughly 2 kg of soil were shaken through a nest of sieves with rectangular holes with an equivalent diameter of 50, 30, and 10 mm and a pan underneath. The aggregate fraction retained on each sieve/pan was oven-dried at 105°C and expressed as a percentage of total dry soil mass. At the time of the analysis, soil water content, measured gravimetrically after the above cited drying was 32% (g g^{-1}), which was considered almost optimal for both soil tillage and for the evaluation of their roughness (Keller et al., 2007). Results were expressed as percentage aggregate size distribution (Van Bavel, 1950). In addition the analysis of the water-stable aggregates before the experiments, obtained using a method already adopted Siegrist *et al.* (1998), highlighted a high level of soil structure (82.4%) confirming the physical (loam texture) and chemical (organic matter) soil fertility.

Seed bank analysis

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

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

Weed seedling emergence evaluation

About 40 days after each of the four soil tillage operations, on the same day as the next tillage, seedling emergence was monitored. Weed seedlings were identified within metal frames (30 cm × 30 cm) placed at the center of the sites (120 sampling points) previously selected for soil extraction. In the control plots where tillage was not performed, seedlings were identified and manually eradicated. This seedling elimination meant that in the following counts, only seedlings that had emerged between two successive soil tillages were considered. The emergence evaluation of each experimental soil tillage type, was carried out on the same days: 20 April, 2 June, 24 July, 8 September and 22 October. In each experimental plot, four sub-plots (0.5-meter squares on each side) were delimited using sticks. In these areas the soil was left undisturbed (no soil tillage was carried out), with manual elimination of the emerged seedlings (on the above-mentioned days of emergence evaluation), in order to quantify the emergence rate in no-till conditions (experimental control).

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

Weed seed weight measurement

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

Calculation of biodiversity of emerged plant community

The data on the total weed seedling emergence, during the experimental period, were used to calculate the biodiversity and dominance of emerged seedlings according to formulas already widely used in phytosociological studies (Benvenuti and Bretzel, 2017). Shannon diversity index (H’) was used to quantify the number of contributing species (species richness) in order to quantify the distribution of individuals between species, and Simpson's index of dominance (D) to measure the probability that two individuals randomly selected from a sample will belong to the same species.

Statistical analysis

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

Results

Seed bank dynamics

Table 1 shows the botanical composition of the seed bank, quantified before the experiments. Over 108,000 seeds m^{-2} were detected, confirming the difficulty of weed management in organic cropping systems. Most of the weed species, about 85% had an annual cycle (therophytes), while a small proportion had a perennial cycle (hemicryptophytes and geophytes). An extraordinary abundance of *Sinapis arvensis* (about 42,000 seeds m^{-2}) were found, which alone accounted for about 40% of the whole seed bank. The other five species detected had a least 4,000 seeds m^{-2}: *Portulaca oleracea* (15,650 seeds m^{-2}), *Echinochloa crus-galli* (12,390 seeds m^{-2}), *Amaranthus retroflexus* (8,525 seeds m^{-2}), *Lolium multiflorum* (7,640 seeds m^{-2}) and *Chenopodium album* (4,330 seeds m^{-2}) with the following percentages (compared to the total): 14.4, 11.4, 7.8, 7.0 and 4.0%, respectively. *P. oleracea*, *E. crus-galli* and *A. retroflexus* have high thermal requirements since they are characterized by a C_4 photosynthetic pathway. A total of 49 species, belonging to 23 different botanical families, were identified.

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

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

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

Finally, the no-till control showed a significantly lower decrease in the final seed bank compared to the initial one. Most species showed less than a 5% decrease and only three poaceae weeds reached a reduction of 10% (*C. dactylon*, *L. multiflorum* and *P. annua*). This trend in tillage efficacy (decreasing from rotary cultivator, rotary harrow, spike tooth arrow and untilled control) was true for nearly all the sampled weeds. However, *P. oleracea* showed that it is a particularly sensitive species to the favourable effect of the crumbling showing a very limited reduction after the spike tooth harrow sequence (only 5.3% and therefore almost unchanged), while this reduction was greater with the rotary harrow (15.4%), and was decidedly higher with the rotary cultivator (35.9%). On the other hand, although *L. multiflorum* was also stimulated to germinate after the soil tillage, it was less dependent on the level of crumbling since the differences between the three types of tillage were decidedly smaller. A similar trend was shown by other poaceae such as *S.viridis, P. annua, Poa trivialis D. sanguinalis, C. dactylon* and *A. myosuroides*, since they were less affected by the soil tillage modalities. Two other poaceae, *E. crus-galli* and *Avena sterilis* were an exception since their seed bank depletion was similar to all the other broadleaved species.

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

Emergence dynamics

The seedling emergence dynamics of the six most abundant weeds (about 85% of the total seedbank) is shown in Figure 6. *A. retroflexus*, *E. crus-galli* and *P. oleracea* showed the highest emergence rates during the month of May (about 40, 35 and 30% respectively) maintaining a high emergence rate already during the following month of June. On the other hand, *S. arvensis* and *L. multiflorum* showed the highest emergence rates at the beginning (April, roughly 50% in both cases) and at the end (October, roughly 35 and 30%, respectively) of the experimental period. *C. album* was in mid-position between these two scenarios. In fact, despite having shown the highest emergence rate at the first sampling carried out in April (roughly 35%), this species maintained a similar emergence in the following month of May (about 30%).

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

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

Seed bank biodiversity

The lack of soil tillage sequence led to germination and emergence in only 22 out of 49 species sampled in the seed bank (Figure 8A). However all the stale seedbed preparations increased the number of species although the degree of increase depended on the soil tillage typology. The number of emerged weed species was about 34 and 42 after the spike tooth harrow and rotary harrow sequence, respectively. Similar results were also confirmed by calculation of the dominance Simpson index (D), with maximum values detected in the untilled control (0.22) and the lowest values detected after the rotary cultivator (0.10) (Figure 8 B). Finally, with the Shannon diversity index (H'), the maximum value was found after the rotary cultivator (1.34), while the rotary harrow and spike tooth harrow showed the lowest values of 0.95 and 0.73, respectively (Figure 8 C). The untilled control showed the lowest value of 0.51.

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

Discussion

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

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

The high biodiversity detected in this experiment was, however, in line with those carried out in other agronomic situations (Boguzas et al., 2004; Legere et al., 2005). This substantial seed bank, together with its marked biodiversity, contributes to an ideal experimental agronomic situation. In fact the aim of the experiments was to verify the effectiveness of diversified strategies based on the pre-existing seed bank. A further favourable agronomic situation was that it rained a little even during the hottest periods of full summer (Figure 2). However, the rain did not hinder the planned schedule (approximately on a monthly basis) of the different soil tillage modalities. The degree of soil cloddiness was strongly related to the type of soil tillage (Figure 3), showing a marked crumbling of the aggregate size with the rotary cultivator. These data are in full agreement with previous experiments that have shown that a rotary cultivator, compared to a rotary harrow, seems to produce less cloddiness in the surface layers (Sandri *et al.*, 1998). The literature also confirms the data on the greater roughness shown by the spike tooth harrow (Salem *et al*., 2015). After the seedbed preparation using the spike tooth harrow, the soil roughness was much higher than after the rotary harrow and even more so after the rotary cultivator.

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

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

Other experiments have shown that the seeds of *P. oleracea* have a higher germination after “zero tillage” than after “minimum tillage” (Chauhan and Johnson, 2009). After long-term “zero tillage” management, most seeds likely concentrate in the upper topsoil due to the extremely low self-burial capacity, and consequently they escape by a depth-mediated burial inhibition.

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

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

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

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

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

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

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

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

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

It is still not clear whether there is a correlation between this germination-inhibition due to the soil clods and the biodiversity reduction of the emerged species. A possible correlation was suggested by the following observation: several of the weed species that were not present, or present in low quantities, as emerged flora in the case of a minor soil crumbling (i.e. spike tooth harrowing) and even more so in the case of the no-till control, had small sized seeds. This suggests that additional data (1,000 seed weight) should be analysed in order to verify whether the size of seeds plays a key role or not. A significant polynomial regression ($p<0.05$) confirmed that small seeds showed a higher soil inhibition since their emergence rate was proportional to the 1,000 seed weight.

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

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

Conclusions

Our experiments clearly showed that the degree of soil crumbling was strongly related to the triggering of the seed bank germination and consequently to the effectiveness of the seedbed preparation. The achievement of about 60% of the emergence rate of the shallowest seed bank (0-10 cm), using the rotary cultivator, is an extremely encouraging result. In addition the deeper soil crumbling was able to even stimulate the germination of small seeds despite their marked tendency to enter dormancy within the soil clods. It is thus crucial to improve knowledge of the seedbed preparation strategies available in terms of the dynamics of both agronomic parameters: seed bank and organic matter. This should lead to the optimal compromise between agronomic positivity and negativity (seed bank depletion and organic matter oxidation respectively) in relation to the choice of the stale seedbed strategy in terms of both: i) typology (rotary cultivator, rotary harrowing, spike tooth harrowing, or others) and ii) frequency.

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

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

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

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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] (%)
Abutilon theophrasti L.	Malvaceae	B	9.23	T	C_3	430	0.40
Alopecurus myosuroides Hudson.	Poaceae	G	1.98	T	C_3	235	0.22
Amaranthus retroflexus	Amaranthaceae	B	0.42	T	C_4	8,525	7.88
Anagallis arvensis L.	Primulaceae	B	0.51	T	C_3	755	0.70
Avena sterilis L.	Poaceae	G	31.2	T	C_3	65	0.06
Bromus sterilis L.	Poaceae	G	9.42	T	C_3	65	0.06
Capsella bursa-pastoris L.Med.	Brassicaceae	B	0.08	T	C_3	80	0.07
Cerastium glomeratum Thuill.	Caryophyllaceae	B	0.05	T	C_3	25	0.02
Chenopodium album L.	Chenopodiaceae	B	0.46	T	C_3	4,330	4.00
Cirsium arvense L.Scop.	Asteraceae	B	1.34	G	C_3	450	0.42
Convolvulus arvensis L.	Convolvulaceae	B	14.5	G	C_3	65	0.06
Conyza canadensis (L.) Cronq.	Asteraceae	B	0.07	T	C_3	55	0.05
Cynodon dactylon (L.) Pers.	Poaceae	G	0.31	G	C_4	140	0.13
Daucus carota L.Scop.	Apiaceae	B	1.12	H	C_3	55	0.05
Digitaria sanguinalis (L.) Scop.	Poaceae	G	0.51	T	C_4	235	0.22
Echinochloa crus-galli L.Beauv.	Poaceae	G	0.87	T	C_4	12,340	11.40
Euphorbia helioscopia L.	Euphorbiaceae	B	2.28	T	C_3	135	0.12
Fumaria officinalis L.	Papaveraceae	B	3.12	T	C_3	345	0.32
Galium aparine L.	Rubiaceae	B	8.81	T	C_3	45	0.04
Geranium dissectum L.	Geraniaceae	B	2.25	T	C_3	75	0.07
Heliotropium europaeum L.	Boraginaceae	B	1.13	T	C_3	35	0.03
Lactuca serriola L.	Asteraceae	B	0.57	T	C_3	15	0.01
Lamium amplexicaule L.	Lamiaceae	B	0.61	T	C_3	35	0.03
Lamium purpureum L.	Lamiaceae	B	0.95	T	C_3	125	0.12
Lolium multiflorum Lam.	Poaceae	G	2.94	T	C_3	7,640	7.06
Malva officinalis L.	Malvaceae	B	5.52	H	C_3	35	0.03
Matricharia chamomilla L.	Asteraceae	B	0.09	T	C_3	320	0.30
Mercurialis annua L.	Euphorbiaceae	B	2.03	T	C_3	75	0.07
Papaver rhoeas L.	Papaveraceae	B	0.14	T	C_3	950	0.88
Picris echioides L.	Asteraceae	B	1.22	T	C_3	155	0.14
Picris hieracioides L.	Asteraceae	B	0.96	H	C_3	120	0.11
Plantago lanceolata L.	Plantaginaceae	B	1.42	H	C_3	85	0.08
Poa annua L.	Poaceae	G	0.28	T	C_3	2,330	2.15
Poa trivialis L.	Poaceae	G	0.12	T	C_3	1,450	1.34
Polygonum aviculare L.	Polygonaceae	B	1.29	T	C_3	1,650	1.52
Polygonum convolvolus L.	Polygonaceae	B	1.48	T	C_3	35	0.03
Polygonum persicaria L.	Polygonaceae	B	2.04	T	C_3	1,850	1.71
Portulaca oleracea L.	Portulacaceae	B	0.11	T	C_4	15,650	14.46
Ranunculus arvensis L.	Ranunculaceae	B	10.2	T	C_3	650	0.60
Raphanus raphanistrum L.	Brassicaceae	B	11.45	T	C_3	75	0.07
Rumex crispus L.	Polygonaceae	B	3.32	H	C_3	355	0.33
Senecio vulgaris L.	Asteraceae	B	0.24	T	C_3	465	0.43
Setaria viridis L.Beauv.	Poaceae	G	2.27	T	C_3	1,120	1.03
Sinapis arvensis L.	Brassicaceae	B	1.82	T	C_3	42,450	39.22
Solanum nigrum L.	Solanaceae	B	0.79	T	C_3	45	0.04
Sonchus oleraceus	Asteraceae	B	0.34	H	C_3	95	0.09
Stellaria media L.Vill.	Caryophyllaceae	B	0.38	T	C_3	385	0.36
Verbena officinalis L.	Verbenaceae	B	0.35	H	C_3	255	0.24
Veronica persica Poiret	Scrophulariaceae	B	1.04	T	C_3	1,335	1.23
					Total seed bank	108,235	100

[1] B= broadleaf; G= grasses

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

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

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)**
Abutilon theophrasti	15.58 a	9.77 b	10.23 b	1.53 c
Alopecurus myosuroides	14.04 a	10.55 c	13.38 a	3.23 d
Amaranthus retroflexus	38.52a	9.95 c	27.06 b	4.32 d
Anagallis arvensis	14.90 a	3,91 b	3.78 b	2.45 c
Avena sterilis	20.00 a	6.15 c	12.31 b	3.21 d
Bromus sterilis	15.38 a	3.08 c	13.85 b	2.28 c
Capsella bursa-pastoris	15.05 a	3.75 b	14.25 a	1.18 c
Cerastium glomeratum	14.92 a	3.04 b	3.34 b	2.62 c
Chenopodium album	33.31 a	15.38 c	26.50 b	8.45 d
Cirsium arvense	12.00 a	5.11 c	7.33 b	3.43 d
Convolvulus arvensis	13.85 a	6.77 c	10.77 b	4.02 d
Conyza canadensis	15.45 a	3.55 b	13.64 a	3.24 b
Cynodon dactylon	15.71 a	11.86 b	13.57 a	10.32 b
Daucus carota	10.91 a	5.45 c	8.49 b	3.45 d
Digitaria sanguinalis	15.49 a	9.79 b	14.04 a	8.87 b
Echinochloa crus-galli	26.21 a	6.87 c	10.90 b	4.45 d
Euphorbia helioscopia	11.11 a	3.70 c	5.93 b	2.32 d
Fumaria officinalis	10.14 a	6.12 b	7.83 b	4.56 c
Galium aparine	13.33 a	7.25 b	11.11 a	6.34 b
Geranium dissectum	10.67 a	5.33 b	6.67 b	2.32 c
Heliotropium europaeum	16.29 a	8.57 c	12.57 b	5.57 d
Lactuca serriola	13.33 a	6.67 b	7.12 b	6.85 b
Lamium amplexicaule	11.43 a	3.71 c	5.71 b	3.58 c
Lamium purpureum	18.40 a	5.60 c	8.40 b	4.43 d
Lolium multiflorum	15.04 a	10.45 b	11.62 b	11.97 b
Malva officinalis	11.43 a	6.67 b	5.71 b	3.45 c
Matricharia chamomilla	14.69 a	1.79 c	3.44 b	1.58 c
Mercurialis annua	14.67 a	7.04 c	9.33 b	6.89 c
Papaver rhoeas	11.58 a	1.79 b	9.32 a	1.65 b
Picris echioides	14.84 a	7.74 b	9.68 b	7.45 b
Picris hieracioides	12.50 a	3.33 c	5.83 b	3.12 c
Plantago lanceolata	11.24 a	3.53 c	5.88 b	3.58 c
Poa annua	19.76 a	10.52 b	17.64 a	10.45 b
Poa trivialis	18.50 a	8.48 b	16.90 a	9.23 b
Polygonum aviculare	10.80 a	3.39 c	6.18 b	4.24 c
Polygonum convolvolus	11.43 a	5.71 b	9.57 a	6.25 b
Polygonum persicaria	13.24 a	9.57 b	10.22 b	6.88 c
Portulaca oleracea	26.26 a	5.36 c	15.40 b	2.24 d
Ranunculus arvensis	18.76 a	6.62 b	6.92 b	5.57 b
Raphanus raphanistrum	14.67 a	11.67 b	12.00 b	9.73 c
Rumex crispus	18.68 a	9.23 c	13.86 b	8.34 c
Senecio vulgaris	12.31 a	6.24 b	6.88 b	4.55 c
Setaria viridis	22.95 a	11.25 c	16.92 b	4.23 d
Sinapis arvensis	35.94 a	18.23 c	25.97 b	4.45 d
Solanum nigrum	18.89 a	16.33 c	12.67 b	2.23 d
Sonchus oleraceus	16.32 a	5.26 b	15.26 a	2.45 b
Stellaria media	22.49 a	12.08 c	16.94 b	5.87 d
Verbena officinalis	14.71 a	1.96 c	7.14 b	1.11 c

Veronica persica	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

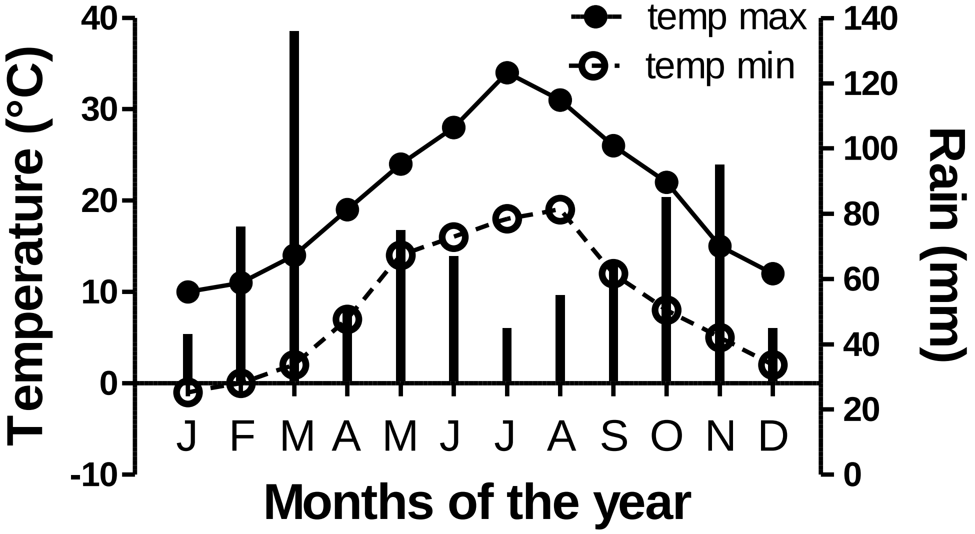

Figure 1. Meteorological data (rainfall, maximum and minimum temperature) occurred during the experimental year 2015

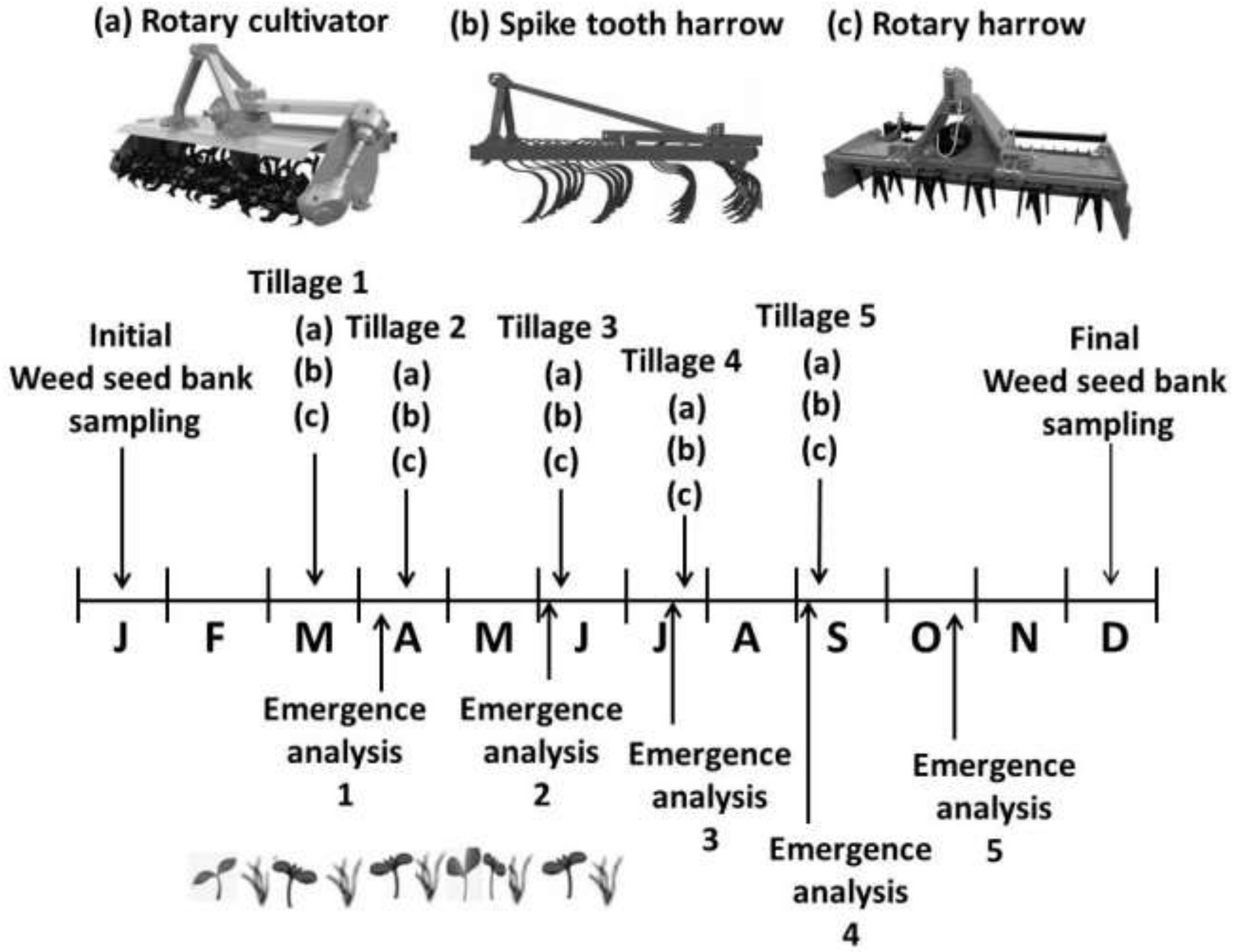

Figure 2. Schematic representation of the soil tillage types and sequence (a= rotary cultivator, b= spike tooth harrow, c= rotary harrow) and times of the experimental evaluations (seedbank and emergence analyses).

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).

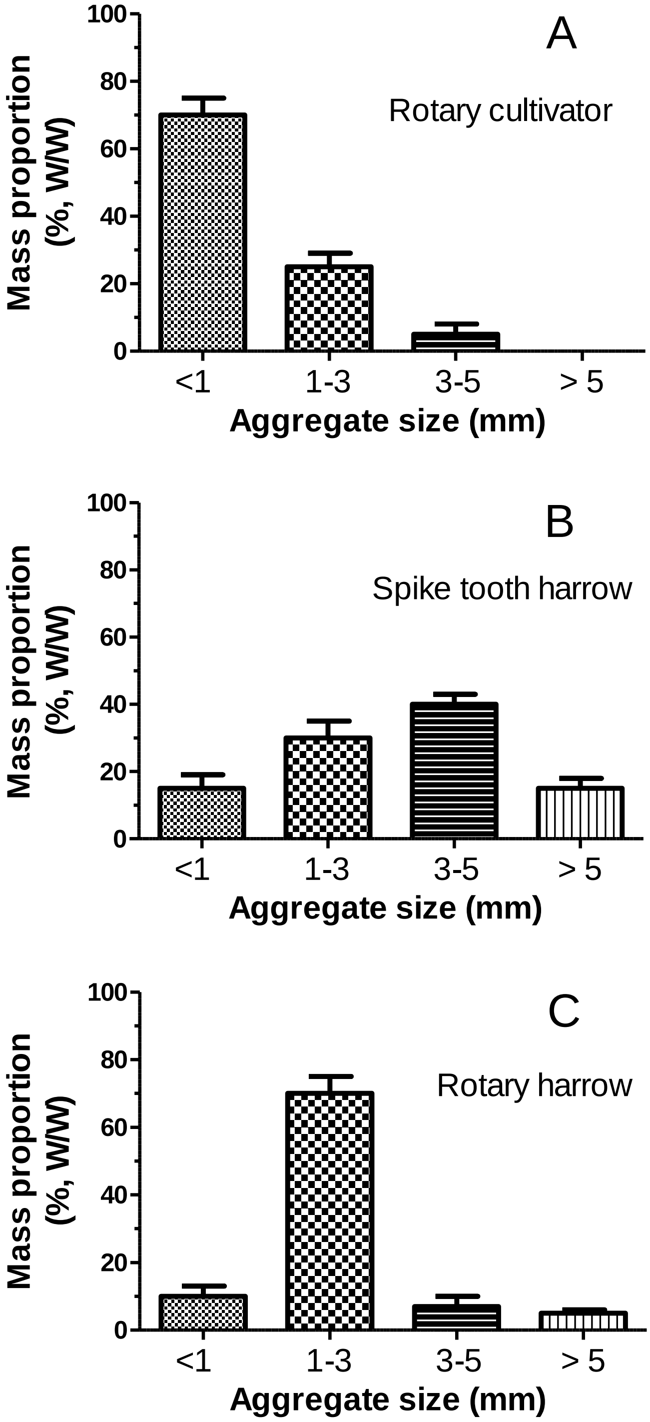

Figure 3. Graphic representation of the dimensional composition of the soil aggregates (mass proportion, % g^{-g}, of the following aggregate size fractions: <1, 1-3, 3-5 and >5 cm) after the diversified tillage. Vertical bars indicate standard errors of the mean.

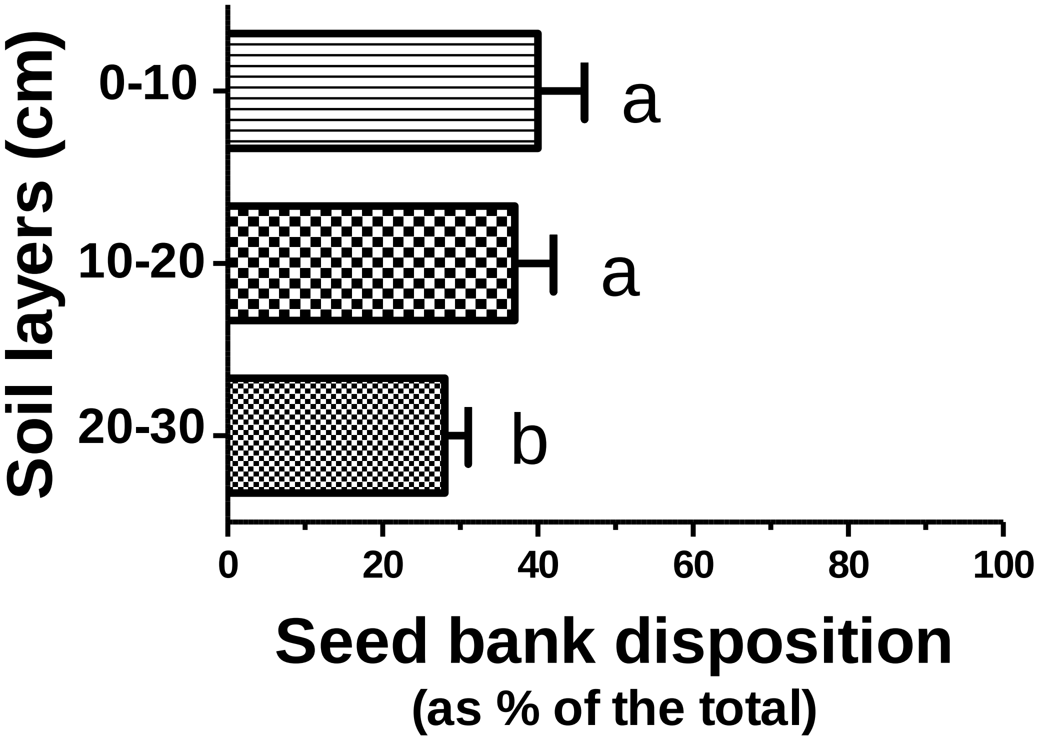

Figure 4. Seed bank disposition in the several soil layers (0-10, 10-20 and 20-30 cm) before the experimental period. Vertical bars indicate standard errors of the mean. Means followed by different letters show statistical difference for $p< 0.05$ according to the Student–Newman–Keuls test.

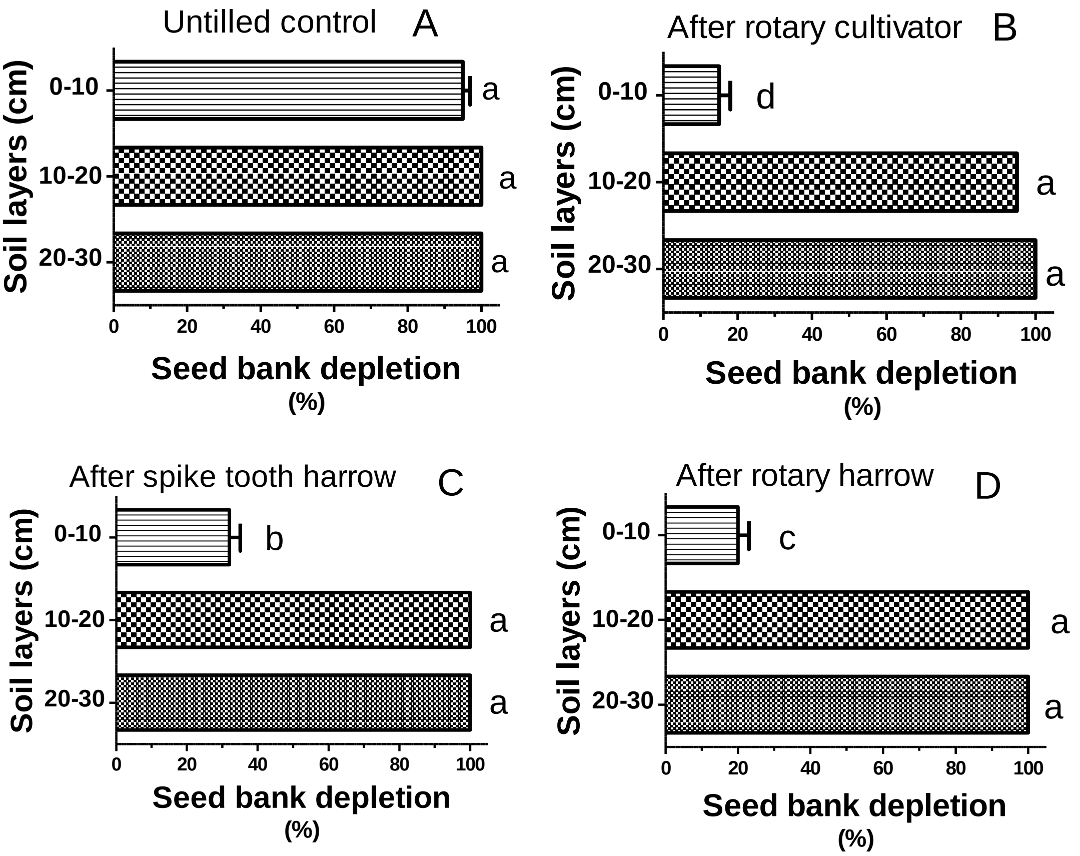

Figure 5. Seed bank depletion expressed as % of the previous seed bank for each soil layer (0-10, 10-20 and 20-30 cm) after differen soil management: untilled control (A), rotary cultivator (B), spike tooth harrow (C) and rotary harrow (D). Vertical bars indicate standard errors of the mean. Means followed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

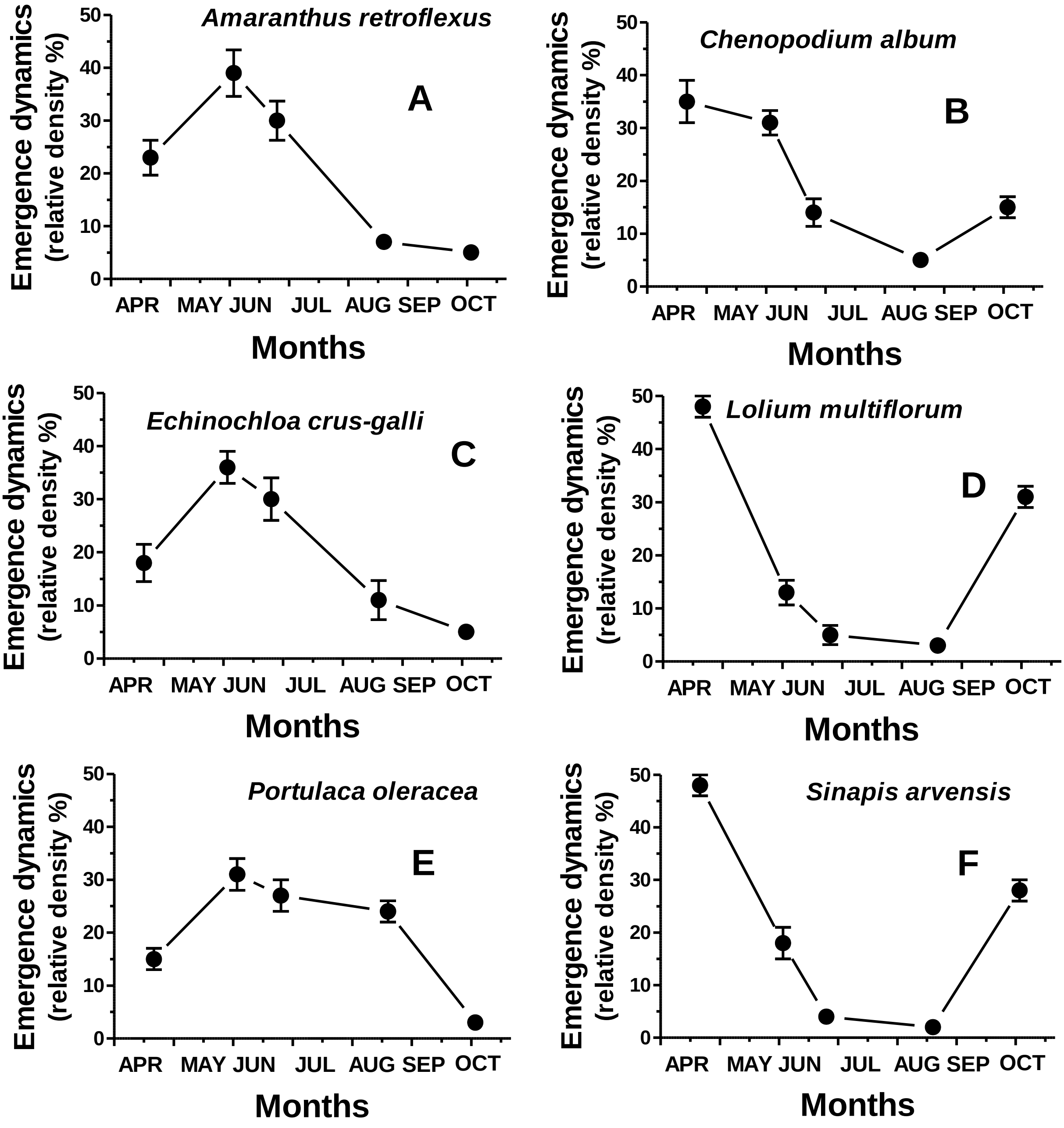

Figure 6. Emergence dynamics during the several months of experimental period (as % of the cumulative emergence) of the six most abundant weed: *A. retroflexus, C. album, E. crus-galli, L multiflorum, P.oleracea* and *S. arvensis*. The data of the different tillage tecniques were pooled due to the lack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

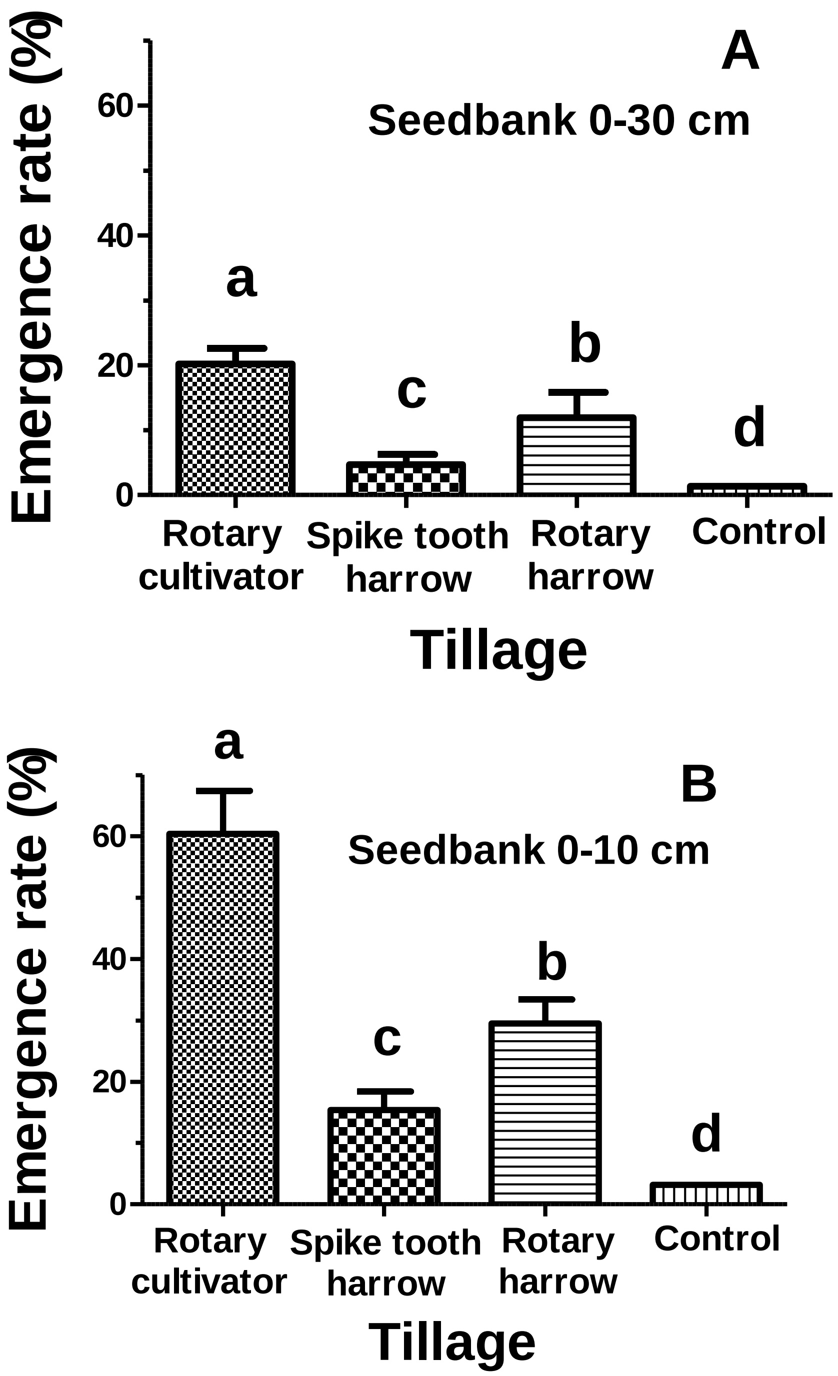

Figure 7. Emergence rate of the different tillage management (rotary cultivator, spike tooth harrow, rotary harrow and undisturbed control) expressed as % referred to the total analyzed seed bank (0-30 cm, A) or referred the only shallowest soil layer (0-10 cm, B). Vertical bars indicate standard errors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

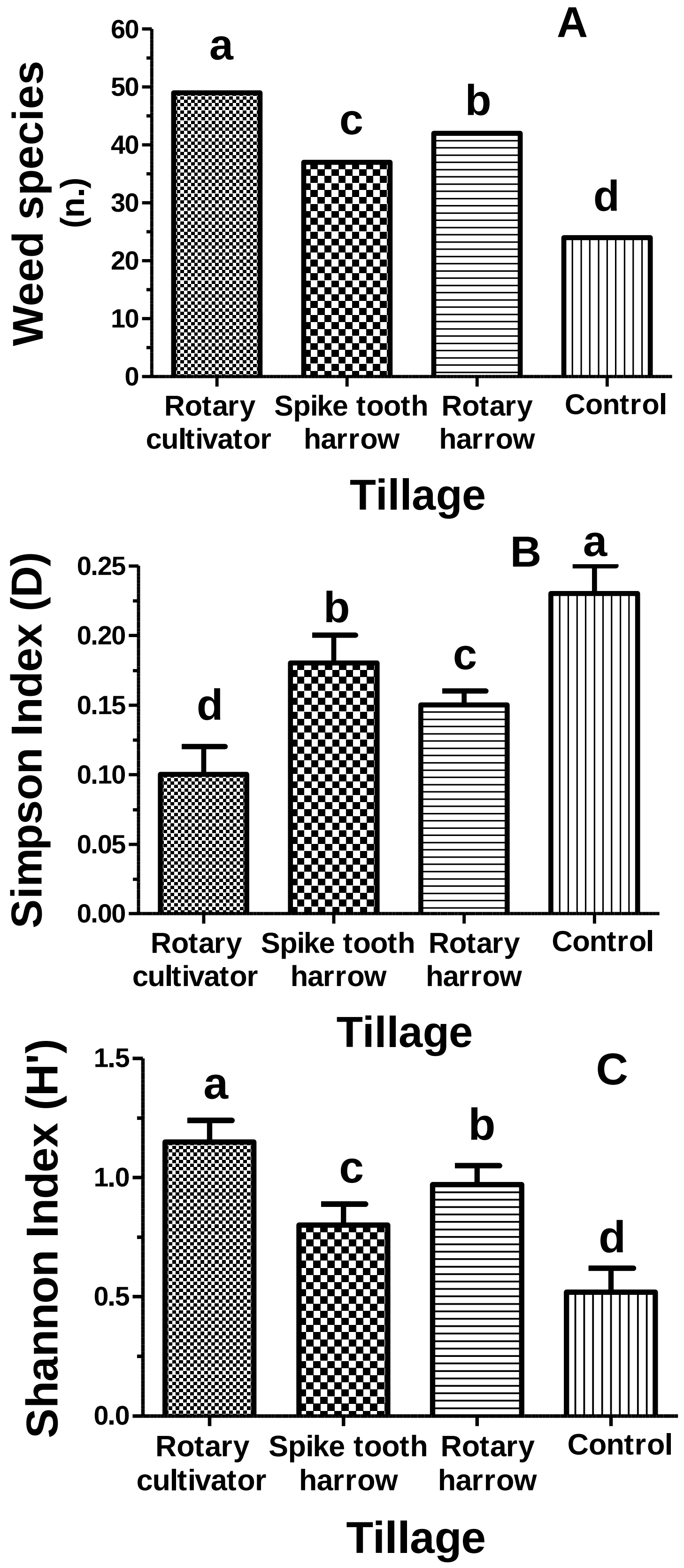

Figure 8. Indexes of biodiversity (Shannon H' (A) and dominance (Simpson, D, (B) and number of emerged weed species (C) as a function of the various tillage managements: rotary cultivator, spike tooth harrow,

rotary harrow and undisturbed control. 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.

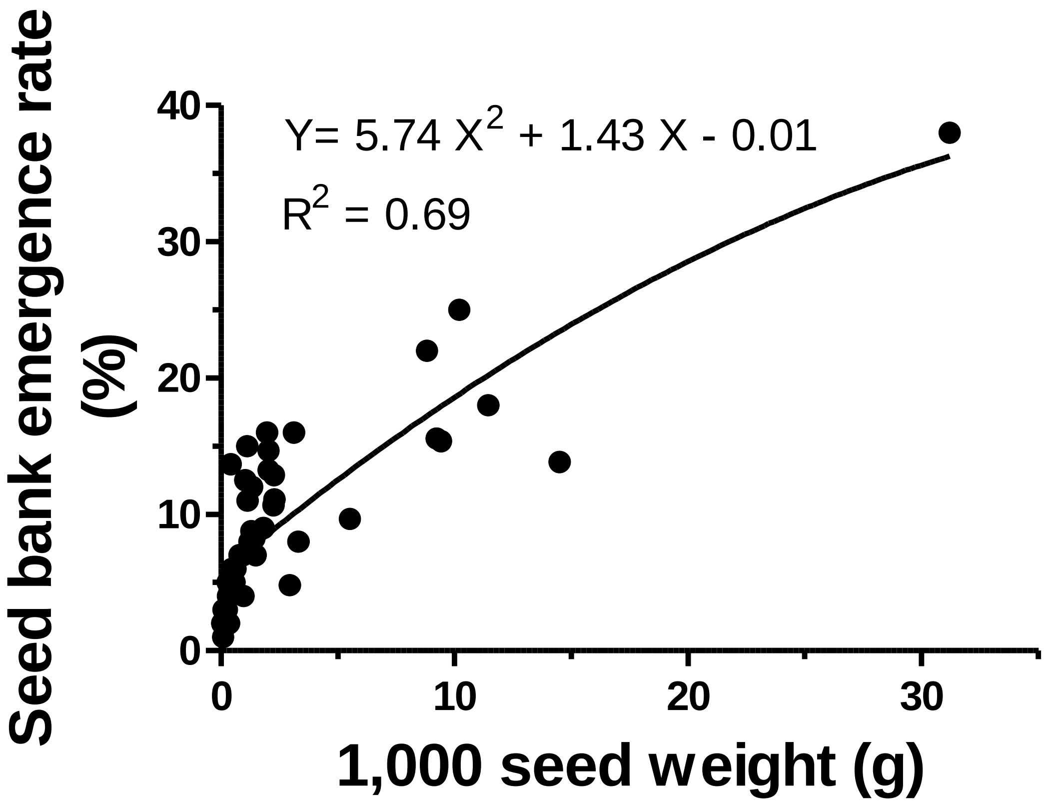

Figure 9. Polynomial regression between seed bank emergence rate (as % of the shallowest soil layer, 0-10 cm) and 1,000 seed weight of the corresponding weed species. The data of the emergence rate are referred only to the stale seedbed preparation carried out by rotary cultivator since this was the only soil tillage capable to trigger germinatin to all of the pre-existing seed bank. The equation (significant for P > 0.05) and the corresponding R^2 value was shown.