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

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Abstract

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

Introduction

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

Tillage can consistently affect the weed community, causing a vertical redistribution of seeds along the soil profile and changes in soil characteristics (which in turn determine changes in soil habitability and, as a consequence, advantage or disadvantage different weed species) and dictating weed control management strategies (e.g., pre-emergence use of nonselective, broad-spectrum, systemic herbicides under no tillage [NT]). Hence, it is not surprising that several studies have shown strong variations in the size and composition of the weed seedbank in response to changing tillage systems [1,5,10]. In general, both the abundance and diversity of soil communities increase with decreasing tillage. In particular, several authors have observed an increase in annual grasses, perennial weeds, and wind-dispersed species under NT [11,12]. Such floral changes under reduced tillage (RT) or NT have been interpreted by some authors as steps in an ecological succession [13,14]. However, other research has shown no alteration of the weed community in response to the application of conservative tillage techniques [15,16].

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

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

A long-term experiment was begun in 1991 to study the effects of the continuous use of conservation tillage techniques (RT and NT) on the performance of crops in cereal–legume rotation systems typical of the semiarid Mediterranean environment. The present paper reports data relative to weed seedbank structure after 18 years of continuous application of the treatments. In particular, we compared the size, composition, and diversity of the weed seedbank between conventional tillage (CT, based on moldboard plowing) and NT within three crop sequences: continuous wheat (W_W), wheat–faba bean (W_F), and wheat–berseem clover (W_B) seed crop.

Materials and methods

Ethics Statement

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

Site characteristics, experimental design, and management

A long-term field experiment was started in the 1991–1992 growing season at Pietranera farm, which is located about 30 km north of Agrigento, Sicily, Italy (37°30’ N, 13°31’ E; 178 m a.s.l.), on a deep, well-structured soil classified as Chromic Haploxerert (Vertisol). Soil characteristics (measured at the beginning of the experiment and referring to the 0- to 0.40-m layer) were as follows: 52.5% clay, 21.6% silt, 25.9% sand, pH 8.1 (1:2.5 H_2O), 1.40% total C (Walkley Black), 1.29 g kg^{-1} total N (Kjeldahl), 36 mg kg^{-1} available P (Olsen), 340 mg kg^{-1} K_2O (exchangeable potassium), cation exchange capacity 35 $cmol_+$ kg^{-1}, 0.38 cm^3 cm^{-3} water content at field capacity (pF 2.5), and 0.16 cm^3 cm^{-3} permanent wilting point (pF 4.5). The climate of the experimental site is semiarid Mediterranean, with a mean annual rainfall of 552 mm, mostly in autumn/winter (74%) and in spring (18%). The dry period is from May to September. The mean air temperature is 15.9°C in autumn, 9.8°C in winter, and 16.5°C in spring. The average minimum and maximum annual temperatures are 10.0°C and 23.3°C, respectively. Weather data were collected from a weather station located within 500 m of the experimental site.

The experiment was set up as a strip-plot design with two replications. Three soil tillage systems (CT, RT, and NT) acted as vertical treatments and three crop sequences (W_W, W_F, W_B) as horizontal treatments. CT consisted of one moldboard plowing to a depth of 0.30 m in the summer, followed by one or two shallow harrowing operations before planting. In the RT plots, primary tillage was chisel plowing to a depth of 0.40 m (noninverting action) in the summer followed by moldboard plowing to a depth of 0.15 m (after the first rains of autumn) and followed by one shallow harrowing operation to prepare a proper seedbed; the moldboard plowing operation was omitted beginning with the eighth year of the experiment (1998–1999). Finally, NT consisted of sowing by direct drilling. The plot size was 370 m^2 (18.5 × 20.0 m). Each year, both rotations (W_F and W_B) were duplicated in reverse order so as to obtain data annually from all crops. In NT plots, weeds were controlled before planting with glyphosate (*N*-[phosphonomethyl] glycine) at a dose of 533–1066 g a.e. ha^{-1}, depending on the development of weeds.

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

Seedbank sampling and analysis

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

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

Calculations and data analysis

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

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

where

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

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

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

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

Results

Type of dispersal, ecophysiological and biological groups, and relative density in the total seedbank of all weeds are shown in Table 1. A total of 46 species were detected during the study, 72% of which were annuals, 9% biennials, and 19% perennials. Tillage system significantly affected the number of weed species in different ways depending on crop sequence (Table 2). For instance, in the upper soil layer (0–5 cm), the number of weed species detected was higher in NT than in CT, with differences between these two tillage systems higher under W_W and W_B than W_F. The opposite was true in the lower soil layer sampled (15–30 cm). In each layer (0–5, 5–15, 15–30, and 0–30 cm), total weed seedling density differed significantly among the three crop sequences in the order $W_W > W_F > W_B$ (Table 3). No variation was observed due to tillage system in the whole layer sampled (0–30 cm); however, in both the upper and the intermediate layers, weed seedling density was significantly higher in NT than CT, whereas in the lower layer the opposite was true. The differences between CT and NT in both the upper and the lower layers were higher in W_W than in W_F or W_B (the crop sequence × tillage system interactions were significant at $P < 0.01$).

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

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

Table 6 shows the seedling density of the primary weed species, which together accounted for almost 90% of the total weed seedlings, regardless of the treatment applied. For most of these species, the effect of tillage system on the total seedling density varied significantly by crop sequence. For instance, the seedling density of *Polygonum aviculare* was higher in CT than in NT, with differences between these two tillage systems higher under W_W than W_F or W_B. The opposite was true for *Papaver rhoeas*. The total seedling densities of *Lactuca serriola* and *Lolium* spp. were higher in NT than in CT under W_B and W_W but not under W_F. The total density of seedlings for *Anagallis arvensis*, was significantly affected by crop sequence (in the order $W_W > W_F > W_B$) and tillage system (NT > CT). Both H_{SH} and E_{SH} were significantly affected by crop sequence, being lower in W_W than in W_F or W_B (Fig. 1), whereas no effect of tillage system was found on these indices.

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

Discussion

Tillage system and crop sequence interacted to determine weed species richness. Regarding this, contradictory results can be found in the literature. For instance, Dorado et al. [5] and Sosnoskie et al. [1] observed greater weed species richness in crop rotations compared to monocultures and when the tillage intensity decreased, whereas Fried et al. [26] found a higher number of weed species in deeply tilled fields compared to those in which NT or minimum tillage were applied. After 12 years of application of four tillage systems in two crop rotations Bàrberi & Lo Cascio [10] found that the number of weed species in the total seedbank did not substantially vary among treatments. They argued that although management practices (such as tillage system and crop rotation) can exert a considerable effect on the emergence and growth of weed species, they can have little or no effect on the reserve of biodiversity in the soil, mainly because of the seed longevity of many weed species, which can serve as a buffer against environmental variability to reduce the risk of extinction. The discrepancy in the aforementioned results shows that the impact of different soil tillage techniques and crop sequences on weed species richness is likely to be highly site specific; this is not surprising given the intrinsic variability in climatic conditions, soil characteristics, management practices, agronomic history, and duration of the experiments. As concerns our experiment, we found that the continuous use of NT, compared to CT, led to a reduction in the number of weed species only under W_W and W_F, whereas under W_B no differences between CT and NT were observed. It is likely that the weed control strategies applied in W_B, based on a spring cut of the vegetation before dissemination of weeds occurred, could have masked the effects of tillage system. As regards the diversity indices, both H_{SH} and E_{SH} were significantly affected by crop sequence, being higher in the two-crop rotations than in the wheat monoculture. This result is in agreement with the findings of Légère et al. [8] and Sosnokie et al. [1]. The crop sequences together with their associated cultural practices (sowing time, weed management strategies, fertilization, etc.) modified the physical, chemical, and biological conditions of the soil, which may have differentially influenced the emergence, growth, and capacity of species to produce seeds, thus modifying their relative abundance. The fact that crop rotations affect the abundance of certain weed species (including some species that are particularly problematic, such as *P. aviculare*) suggests that such agronomic practice can be essential when planning efficient weed control strategies. As concerns tillage system, although it modified the weed composition by altering the relative abundance of many species, it did not influence either H_{SH} or E_{SH}. This result is in line with the findings of Légère et al. [8], who observed that tillage had little effect on weed diversity indices but played an important role in determining the composition of the weed community.

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

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

Both tillage system and crop sequence led to qualitative changes in weed flora. The continuous use of NT led to an increase in eight species—*P. rhoeas, A. arvensis, V. hederifolia, L. serriola, Phalaris* spp., *Lolium* spp., *E. elaterium,* and *P. oleracea*—even if, for most of these species, the magnitude of the differences between the two tillage systems was markedly affected by crop sequence. Other authors have found a progressive increase in most of these species due to a reduction in tillage intensity [5,10]. These species differ markedly in their ability to compete with crops; for instance, *A. arvensis* is not aggressive [30], whereas *L. serriola* is particularly competitive and thus able to cause considerable losses in yield. From an agronomic point of view, the increased abundance of this species represents a serious weed management concern for NT cropping systems. For many species, particularly for *P. rhoeas*, the superiority of NT over CT (in terms of seedling density) was less under W_B with compared to both W_W and W_F. This result is probably attributable to the weed control strategy adopted during the berseem clover growing season (i.e., the spring cut of the vegetation) which drastically reduced the probability of seed dissemination for many weed species, thus masking the effect of tillage system in the W_B crop sequence. The severe reduction in seedling density of many weed species in W_B probably led to a release of ecological niches that were then occupied by other species. This may have been true for both *P. oleracea* and *V. hederifolia*, whose density increased only in the NT–W_B system. This result can be explained considering the growth habit of these two species (which is prostrate or semi-prostrate), a trait which probably allowed plants to avoid the cut made during the berseem clover growing season, thus increasing the probability of their seeds being disseminated. As concerns monocotyledons, the increases in seedling density observed for *Phalaris* spp. and *Lolium* spp. in NT systems were particularly pronounced under W_W. These two species are favored by the cessation of weed control by superficial soil disturbance [14,31,32]. Moreover, in our experiment, the 18-year continuous application of wheat monoculture resulted in conditions favorable to these two species also due to the use of herbicides on wheat, which are less efficient on monocotyledons than on eudicotyledons [33]. With regard to *Phalaris* spp., other studies have found opposite results from ours, underlining a higher density of *Phalaris* spp. in CT than in conservative tillage systems [34,35]. Taylor et al. [36] found that peak emergence of *Phalaris paradoxa* seedlings was in the middle of the winter cropping season in NT plots but at the beginning of the cropping season in cultivated plots. This altered periodicity of the emergence of *Phalaris* spp. seedlings can make it easier to control seedlings in CT plots through pre-sowing operations; this in turn could lead to a progressive decline in *Phalaris* spp. seeds in the seedbank compared with plots that receive no cultivation. In our experiment, *P. aviculare* showed a preference for moldboard plowed soil, a result that is in agreement with Dorado & López-Fando [34]. For this species germination is markedly affected by both fluctuations in soil moisture conditions experienced by seed during dormancy and the sensitivity of seed to light [37], both factors that are modified by tillage system. As regards *R. segetum* and *S. arvensis*, an increase in viable seeds in CT compared with NT was observed in the W_F crop sequence only. Other authors have highlighted a preference of *S. arvensis* for moldboard plowed soil [7], whereas no information is available for *R. segetum.* In the present experiment, the differences in seedling density between CT and NT as the crop sequence changed are attributable to the different weed control methods adopted in the three crop sequences. The spring cut of berseem clover and the herbicides applied on wheat effectively controlled both *R. segetum* and *S. arvensis,* whereas mechanical control (shallow hoeing) performed during the faba bean growing season did not always guarantee efficient control, thus allowing these two species to disseminate.

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

The CDA allowed us to discriminate among the different cropping systems, highlighting how the interaction of the treatments applied (tillage system and crop sequence) affected the weed seedbank in both quantitative and qualitative terms. The presence of berseem clover in the crop rotation markedly influenced the composition of weed community, masking, at the same time, the effects of tillage system. In contrast, NT exerted great pressure on weed communities in both the W_W and W_F cropping systems, which were plotted near each other and distant from all other treatments, whereas crop sequence (W_W *vs* W_F) differentiated weed community composition only under CT. Some authors [13,14] have offered an ecological interpretation of weed flora dynamics under different tillage systems. In particular, Zanin et al. [14] reported that a reduction in the mechanical disturbance of soil can result in marked changes to flora, with a tendency for weeds to undergo succession toward a more mature community, with an increased importance of biennial, hemicryptophytes, and geophytes species. Other studies have highlighted the fact that a reduction in soil disturbance generally results in an increase in the occurrence of perennial weeds in many arable cropping systems [40,41]. Although biennial weed species were generally favored in NT systems in our study, the data did not allow us to clearly demonstrate the existence of a gradient reflecting ecological community succession. The effects of tillage system probably could have been reduced or masked by other agronomic factors, as observed by Derksen et al. [42] and Légère et al. [8] in other studies.

In conclusion, this weed seedbank analysis performed within a long-term (18-year) field experiment has provided useful information about the effects of some agronomic practices on weed population dynamics in wheat-based Mediterranean cropping systems. Our results suggest that crop rotation and tillage technique both act as filters that determine (and, moreover, often interact with each other in determining) the composition and abundance of weed species in the soil seedbank. In particular, compared with crop rotations (wheat–faba bean and, particularly, wheat–berseem clover), the continuous monoculture of wheat resulted in a great increase in total weed seedbank density and, at the same time, a reduction in weed diversity, with a strong increase in some species, some of which are potentially hard to control. In contrast, tillage system had no effect on the size of the weed seedbank but significantly modified its composition as well as the distribution of weed seeds along the soil profile. Indeed, the adoption of a CT technique (based on moldboard plowing) favored some weed species (mainly *P. aviculare*); in contrast, the continuous use of NT led to an increase in weed seeds in the upper soil layer and, moreover, resulted in a significant increase in the seed density of some problematic species, such as *P. rhoeas, Phalaris* spp., and *L. serriola.* In any case, the effects of tillage system on weed seedbank size and composition were enhanced in both the wheat–faba bean cropping system and the continuous monoculture of wheat but weakened in the wheat–berseem clover cropping system. Hence, knowledge on how agricultural practices influence the weed community in the mid and long term, from both a quantitative and qualitative point of view, may enable experts to predict the spread of problematic weeds and to plan efficient control strategies that favor the development of a weed community with little impact on the agroecosystem. From a practical point of view, these results suggest that, although NT is environmentally friendly because it mitigates soil erosion, reduces energy use, and enhances wildlife habitat, farmers should only apply such a conservative technique within an appropriate crop sequence.

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Figures legends

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

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

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

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

46	*Veronica persica*	Th	In	An	Baro	0.33	+	+	+	+

Th, therophytes; H2, biennial species; G, geophytes; Hr, hemicryptophytes. Au, Au/Wi, Wi/Sp, Sp, Sp/Su, Su: autumn, autumn/winter, winter/spring, spring, spring/summer, summer germinating species. In, indifferent (species germinating in any month). An, annual species; Bi, biennial species; Per, perennial species. Baro, barochory; Anem, anemochory; Zooc, zoochory; Zooc/Anem, zoochory/anemochory.

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

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

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

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

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

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

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

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

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

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

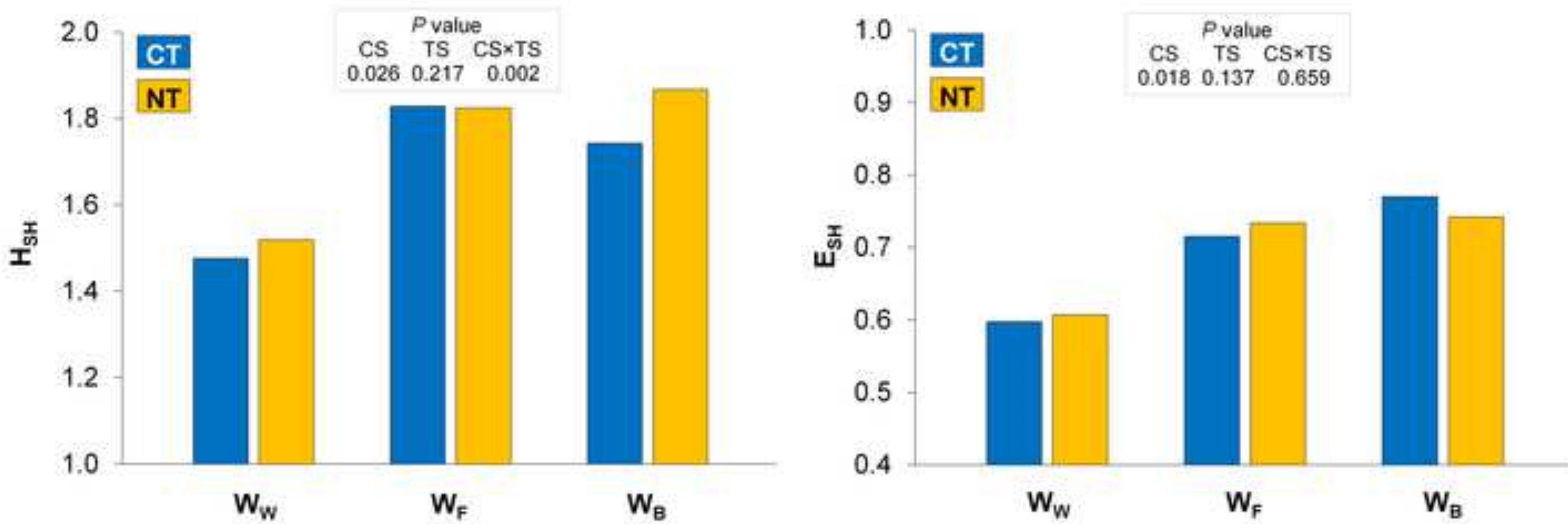
	W_W		W_F		W_B		*P*-value		
	CT	NT	CT	NT	CT	NT	CS	TS	CS × TS
Autumn	1.1	1.9	1.1	0.6	1.1	0.3	0.143	0.796	0.005
Autumn/Winter	0.2	0.5	2.0	1.1	0.6	0.3	0.142	0.537	0.429
Winter/Spring	29.5	18.1	13.3	15.7	8.9	2.9	< 0.001	0.121	0.055
Spring	13.4	18.3	5.7	9.3	1.8	8.4	< 0.001	0.001	0.216
Spring/Summer	0.5	0.4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

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

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

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

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



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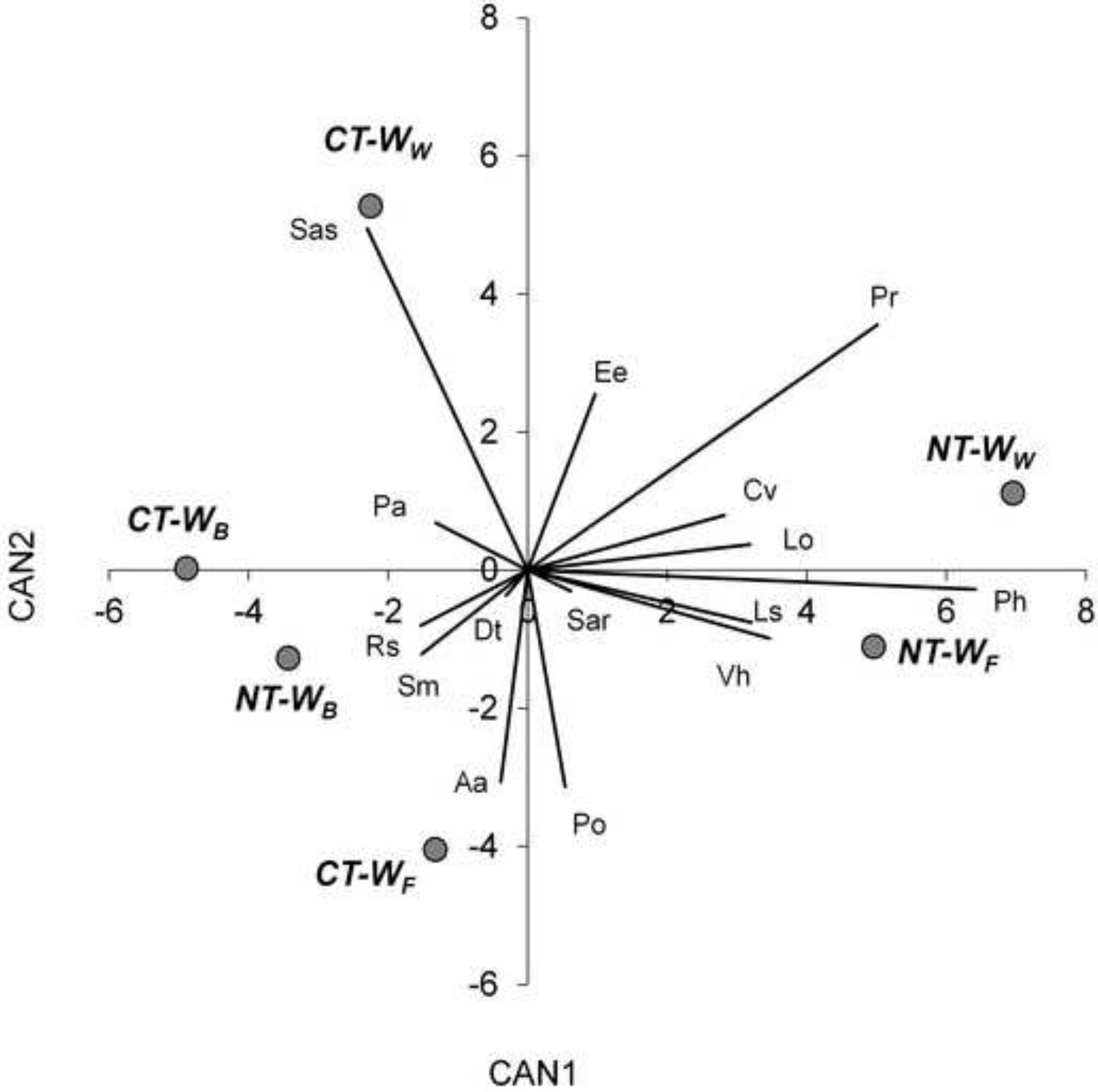

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