

## Long-Term Management Affects the Community Composition of Arable Soil Seedbanks

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The intensification of farming practices has reduced weed infestations, but it has also led to a reduction in weed diversity and changes in species composition. These effects are well described for aboveground flora; however, it is less clear how these effects might be expressed in the soil weed seedbank. We evaluated the effects of different long-term farm management strategies on the weed seedbank abundance, diversity, and community composition in the DOK (bioDynamic, bioOrganic, and Konventionell) field trial established in 1978 at Therwil, Switzerland. The trial compares biodynamic, organic, and conventional farming systems, which mainly differ in fertilization, weed control strategies, and pest control. The species richness and seed abundance of the weed seedbank were higher in the organic and biodynamic systems compared with the conventional ones. The different farming systems favored shifts in species assemblages, because specific management practices, such as herbicide application and type of fertilization, acted as filters that selected against certain species but promoted others that were more adapted.

**Key words:** Fertilization, herbicide application, seed abundance, species richness, weeds.

Knowledge regarding arable weed seedbanks is of great importance, because seedbanks represent a more reliable estimate of weed communities than standing vegetation, as the latter is more affected by current management and by the environmental stochasticity of a given year (Albrecht 2003; Hawes et al. 2010). Weed density, species richness, and the composition of arable weed seedbanks are assumed to reflect medium- and long-term cumulative field management, because they are the result of the effects of processes that occurred in the past (Albrecht 2003, 2005; Menalled et al. 2001) and of the effects of present weed management on weed survival and reproduction (Cavers and Benoit 1989). Weed seedbanks might represent a reserve of diversity with the potential to restore species of a previous community (Hawes et al. 2010; Potts et al. 2010), which is of major importance considering concerns over the conservation of species diversity in arable fields (Hyvönen and Salonen 2002; Robinson and Sutherland 2002; Sutcliffe and Kay 2000). The

motivation for the recovery and conservation of arable weed diversity lies in the fact that it represents an essential part of our natural heritage related to the land-use history, besides its key functional role providing alternative resources for pollinators, herbivores, and granivorous animals and habitat and refuge for some crop-associated fauna (Altieri 1999; Clergue et al. 2005; Marshall et al. 2003). Additionally, weed seedbanks are the primary source of weed establishment in arable fields, and they provide stronger estimates of future weed problems than measures of aboveground vegetation (Bàrberi et al. 1998; Izquierdo et al. 2009). Hence, the consideration of the diversity and composition of soil seedbanks may be used to ascertain appropriate farm management to optimize diversity while controlling future weed infestations (Albrecht 2003).

The high rates of herbicide and fertilizer applications that characterized crop production systems since the second half of the last century have affected weed seedbanks (Robinson and Sutherland 2002; Ryan et al. 2010). For instance, herbicide application is considered the most effective method in reducing weed infestations in fields and decreasing the abundance of weed seedbanks (Bàrberi et al. 1998). However, it also results in reduction in species diversity, particularly of those species that are herbicide susceptible (Bàrberi et al. 1998; Cavers and Benoit 1989; Hawes et al. 2010; José-María and Sans 2011; Robinson and Sutherland 2002; Ryan et al. 2010; Squire et al. 2000), but promotes those species that are able to develop resistance to

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herbicides (Heap 2009). This may influence the community assembly toward species of less conservation interest. Fertilization can also affect the diversity and the composition of weed seedbanks (De Cauwer et al. 2010). Higher doses of fertilizers may promote more nitrophilous and competitive weed species, which have the ability to take up nutrients faster (Moonen and Bàrberi 2004; Robinson and Sutherland 2002). The affinity of species to nitrogen levels has been quantified by Ellenberg (1991) and may allow relating the ecological preferences of species with the availability of nitrogen. The nature of fertilizer inputs, which differ in the rate of nutrient release, may also represent a potential filter during the assembly of weed communities (De Cauwer et al. 2010). Hence, both herbicide application and fertilization may act as assembly filters that select against those species with less adapted traits (Albrecht 2003; Menalled et al. 2001; Ryan et al. 2010).

Integrated or organic farming systems were developed to minimize the environmental impacts of agricultural practices. The same intention drove the emergence of biodynamic agriculture, which utilizes specific fermented herbal preparations as compost additives and field sprays to improve soil and crop quality and hasten composting in addition to the common tools of organic agriculture (Carpenter-Boggs et al. 2000; Zaller and Köpke 2004). Since farm management in all these systems tends to be less intensive than in the conventional ones, because of less or no application of herbicides and limited mineral fertilizer inputs, they do sustain higher arable weed diversity, even in the soil seedbank (Albrecht 2005; Boguzas et al. 2004; Ryan et al. 2010; Squire et al. 2000). However, they usually involve higher weed density as well (Hawes et al. 2010), which may affect crop yields.

The aim of this study was to evaluate the effects of different long-term farming systems on weed seedbank abundance and diversity and on the community composition to acknowledge the impact of past and present management on what could become the future aboveground weed community. The study was performed in the DOK (bioDynamic, bio-Organic, and Konventionell) long-term experiment set up at Therwil, Switzerland, in 1978, with the aim of comparing biodynamic, organic, and conventional farming systems. Long-term agroecosystem experiments are fundamental to study crop production, nutrient cycling, and environmental impacts of agriculture (Rasmussen et al. 1998). However, only a few long-term field experiments spread all over the

world are devoted to studying organic farming systems (Drinkwater et al. 1998; Mäder et al. 2002; Raupp 2001), and DOK is one of the longest running in the world. The DOK trial has been a platform for several studies addressing the effects of different farm management strategies on crop yields, soil fertility, and diversity of soil microbes, earthworms, arthropods, and aboveground weeds (Hartmann et al. 2015; Mäder et al. 2002, 2006). However, the long-term effects of different farming systems on the soil seedbank have not yet been addressed, despite being of interest in long-term trials due to the soil seedbank's expected response to long timescale management and its relation with future weed communities.

Specifically, in this study, we assessed the effects of biodynamic, organic, and conventional farming systems on weed seedbank abundance, diversity, and community composition. Moreover, we specifically assessed whether herbicide and fertilizer applications may have acted as filters on the community composition of the weed seedbank over the course of the long-term cropping systems.

## Materials and Methods

**Study Site and Experimental Design.** The study was conducted in the DOK trial (Therwil, Switzerland; 47.50°N, 7.55°E), a long-term agricultural experiment established in 1978 by the Agroscope Reckenholz-Tänikon research station and the Research Institute of Organic Agriculture. This trial compared five different farming systems: two conventional systems managed according to the guidelines for integrated plant protection since 1985 [conventional (hereafter CONFYM) and mineral (CONMIN)]; two organic farming systems [organic (BIOORG) and biodynamic (BIODYN)]; and an unfertilized (NOFERT) farming system (Mäder et al. 2002). The mean annual temperature of the trial site was 9.5 C, and the mean annual precipitation was 785 mm.

Crop rotation and the primary soil tillage, which are typically a compromise between the optimal agricultural practices for organic and conventional management systems, were identical in the five farming systems of the trial. A primary soil tillage to prepare the seedbed and control weeds was conducted with a moldboard plow at 20 cm deep in all farming systems. The 7-yr crop rotation corresponding to the fifth rotation period of the trial (2006–2012) included a sequence of potato (*Solanum tuberosum* L.), winter wheat 1

(*Triticum aestivum* L.), soybeans [*Glycine max* (L.) Merr.], maize (*Zea mays* subsp. *mays* L.), winter wheat 2, grass-clover 1 (3% red clover [*Trifolium pratense* L.], 11% white clover [*Trifolium repens* L.], 14% orchardgrass [*Dactylis glomerata* L.], 8% red fescue [*Festuca rubra* L.], 8% timothy [*Phleum pratense* L.], 28% perennial ryegrass [*Lolium perenne* L.], 28% Kentucky bluegrass [*Poa pratensis* L.]), and grass-clover 2 (9% red clover, 12% white clover, 15% orchardgrass, 36% meadow ryegrass [*Lolium pratense* Huds.], 8% timothy, 18% perennial ryegrass, 28% Kentucky bluegrass), which were temporally shifted in three parallel subplots for each farming system. Therefore, each year, three different crops were grown for each farming system. The sampling was performed in 2009, when the crops were maize, winter wheat 2, and potato. The current study was conducted on the potato plots.

The experiment was designed as a randomized block consisting of four blocks (field replicates), each one with all the combinations of the five farming systems for each crop planted each year (Fließbach et al. 2007). A single plot for each crop and farming system was 5 by 20 m. There was a buffer zone strip of 6 m planted with grass and regularly mulched between the BIOORG, BIODYN, CONFYM, and CONMIN experimental plots. CONMIN and NOFERT plots were directly adjacent to each other. Nevertheless, 2 m distances were left between all of the sampled plots. Detailed information about the DOK long-term trial is given in Mäder et al. (2002) and Fließbach et al. (2007).

**Management Systems.** The farming systems differed mainly in terms of fertilization and plant protection strategies. The organic systems (BIOORG and BIODYN) were fertilized with farmyard manure and slurry corresponding to 1.2 livestock units ha<sup>-1</sup> (for the first and second rotation periods, from 1978 to 1991) and 1.4 livestock units ha<sup>-1</sup> (from the third rotation period onward). While only partially decomposed manure was used in the BIOORG plots, composted farmyard manure was added to the BIODYN plots. BIOORG plots were also fertilized with mineral fertilizer based on small amounts of rock dust containing minerals and trace elements, and potassium magnesia, but these were only 30 to 35% of that used in the CONFYM system. The conventional CONFYM system was fertilized with the same amount of farmyard manure as the organic farming systems and with mineral fertilizers up to the recommended dose of fertilizers of the plant-specific Swiss standards. The

mineral-fertilized plots (CONMIN) were not fertilized during the first crop rotation, but they were then amended exclusively with mineral fertilizers up to the recommended dose. The mean annual input of total nitrogen, phosphorus, and potassium to the organic systems was 60 to 65% of the input of the conventional systems during the period from 1978 to 2005 (Mäder et al. 2006). In summary, the BIODYN, BIOORG, and CONFYM systems represent mixed farms with arable land and livestock with increasing rates of fertilization, and the CONMIN system mimics a conventional system without livestock. The nonfertilized systems did not receive any type of fertilizer from the establishment of the trial.

The concept of plant protection differs between organic and conventional systems. Weed control in organic systems and in the NOFERT system was only mechanical, while chemical and mechanical weed control was carried out in conventional systems. The postemergence weeding was conducted either by harrowing or manual removal in the organic and nonfertilized farming systems, depending on the crop-sowing pattern. One to three herbicide sprays were applied per crop in the conventional systems, except when grass-clover was sown. For disease control, a decoction of field horsetail (*Equisetum arvense* L.) was applied in the BIODYN farming system to wheat and potatoes, and silica-rich rock dust was applied to potatoes in both organic systems. *Phytophthora infestans* (Mont.) de Bary (late blight) in potatoes was additionally controlled by reduced doses of copper in BIOORG plots. For this process, up to eight fungicide sprays were applied in both conventional systems to potatoes based on economic threshold values, while one to two fungicide applications were performed in conventional wheat production. Biocontrol (*Bacillus thuringiensis* subsp. *tenebrionis*) was employed to regulate pests [Colorado beetle, *Leptinotarsa decemlineata* (Say)] in organic plots. In conventional plots, one to three chemical insecticides were applied to potatoes and wheat based on threshold values. In addition, as specific treatments, biodynamic preparations of cow manure and silica were applied in the BIODYN and in NOFERT plots, and plant growth regulators were applied to wheat in conventional farming systems. Manure compost and slurry used in the BIODYN system were amended at the beginning of composting by processed herbs of common yarrow (*Achillea millefolium* L.), wild chamomile (*Matricaria recutita* L.), stinging nettle (*Urtica dioica* L.), English oak (*Quercus robur* L.),

dandelion (*Taraxacum officinale* G. H. Weber ex Wiggers), and common valerian (*Valeriana officinalis* L.) (Carpenter-Boggs et al. 2000, Zaller and Köpke 2004).

**Sampling.** Sampling was performed in June 2009 in the potato crop plots. Thirty-five 2.8-cm-diameter by 20-cm-deep soil cores were collected on a grid pattern of 5 by 7 soil cores separated 0.75 by 2 m. This grid pattern was placed in the inner 12 by 3 m of each plot to avoid the edge effect. The 35 soil cores from each plot were evenly distributed in five aluminum trays (seven soil cores of each line in each tray) and placed in an unheated greenhouse bench at the Faculty of Biology, University of Barcelona. They were placed into a dark 2 to 4 C refrigerator for a week to aid in breaking dormancy of some weed seeds. Then, they were kept under a natural photoperiod and watered regularly. The bench was covered with a mosquito net to prevent the invasion of seeds from the surrounding area. The position of the trays was randomized every 3 to 4 wk. Samples were periodically allowed to dry mildly, and they were then turned, aerated, and watered again to stimulate germination. A detailed description of the method was given by Gibson (2002). Emerged seedlings were periodically identified and removed after being counted. Thirteen censuses were conducted from June 2009 to December 2010. The nomenclature followed Tutin (1993).

**Diversity Analysis.** The total number of weed species in the soil seedbank for each farming system was obtained by summing the species from all of the censuses. The abundance of each species was assessed as the number of seedlings per square meter, which was obtained by dividing the total number of seedlings emerged in each aluminum tray by the total area of soil cores per tray ( $[(0.014)^2 \times \pi] \times 7$ ). The diversity of the weed seedbank was assessed as the total number of species identified in each tray, considered as soil samples thereafter.

The effect of the farming system on the total weed seedbank abundance and diversity was analyzed by linear mixed-effects models (Pinheiro and Bates 2000). Farming system was included as the fixed factor and the field replicate and plot as random factors. The soil sample was considered nested to plot, which was in turn nested to field replicate to take into account the nonindependence. The normality and homogeneity of variance of the residuals were tested visually and by using the Shapiro-Wilk test and the Levene test, respectively.

Abundance data were log-transformed to meet the assumptions of normality and homoscedasticity of the residuals. Orthogonal contrasts, selected a priori to check for significant differences between interesting comparisons, were performed to compare the different levels of the farming systems. NOFERT plots were compared with the other systems that were fertilized; organic farming systems (BIOORG and BIODYN) were compared with the conventional ones (CONFYM and CONMIN); and BIODYN was compared with the BIOORG farming system and CONFYM with CONMIN. Statistical analyses were conducted with R (v. 2.12.1, R Development Core Team 2010), using the package “lme4” (Bates et al. 2008) for mixed-effects models and “lmerTest” to evaluate the significance (Kuznetsova et al. 2014).

**Seedbank Composition Analysis.** The effect of the different farming systems on the species composition of the seedbank was analyzed through a multivariate analysis based on presence/absence data. Species that only appeared once (eight species) were removed, because they were not informative for the classification. Thus, the Jaccard dissimilarity index was computed among the 42 species for each soil sample. Nonmetric multidimensional scaling (NMDS) analysis, using a stable solution by random starts, was performed with  $k = 2$  dimensions to facilitate the graphical ordination. The farming system factor was plotted onto the ordination at the corresponding centroid position obtained during the classification. The significance of the effect of farming system on the ordination was tested with a random permutation multivariate ANOVA using distance matrices. This analysis allows partitioning distance matrices among sources of variation and fitting linear models to the distance matrices. Again, we used the Jaccard dissimilarity index, and species present in only one sample were removed. The significance of the farming system was obtained by means of F-tests based on sequential sums of squares from permutations of the raw data, restricting permutations within each plot so as to take into account the hierarchical design (Legendre and Anderson 1999). The most frequent species appearing in more than 20 soil samples (19 species) were also plotted onto the ordination at the positions obtained in the classification. We carried out this ordination analysis under R (v. 2.12.1, R Development Core Team 2010) using the package “vegan” (Oksanen et al. 2013).

**Evidence for Community Assembly Filters.** The nitrogen Ellenberg indicator value (Ellenberg 1991), which is an index based on classification of plants according to their ecological niche position along an environmental gradient, was used to check for the filtering effect of nutrient availability on the weed community assembly. We calculated the weighted average nitrogen indicator value for each soil sample by adding the relative abundance of each species and its nitrogen index value. Therefore, higher nitrogen indicator values indicate higher nitrogen affinity. Species for which we were unable to assign a nitrogen Ellenberg indicator value were excluded from the analysis. This was either because they were not identified at the species level and the species of the genera differed in this value (four species out of 50) or because no information was available (two species out of 50). Data on herbicide-resistant species were used to test for the effect of herbicides on weed assembly (Heap 2009). Species were classified into two categories according to any known herbicide resistance reported (Y) or not reported (N). Species identified at the genus level were excluded from the analysis (five species out of 50). The relative importance of herbicide-resistant species was calculated as the relative abundance of species with herbicide resistance for each soil sample. The effect of the different farming systems on the assembly of the nitrogen indicator values and of the herbicide resistance of the weed community was analyzed using the same approach and statistical analyses as in the diversity analysis.

## Results and Discussion

**Seedbank Overview.** Overall, we recorded 2,048 seedlings in the NOFERT system soil samples, whereas 501 and 517 seedlings were counted in the CONFYM and CONMIN systems, respectively, and 1,700 and 1,224 seedlings were counted in the BIOORG and BIODYN systems, respectively. Thus, a total of 5,990 seedlings were counted among all of the farming systems, corresponding to 50 weed species. Twenty-seven species and 34 species appeared in the CONFYM and CONMIN systems, respectively. Thirty-seven species and 36 species were found in the BIOORG and BIODYN systems, respectively. Thirty-five species were counted in the NOFERT system. Twenty-three species were recorded in all of the farming systems, and only a few species were found exclusively in one farming system. For example, 10 species were exclusive to the organic systems (BIOORG and/or BIODYN), whereas only

three species were exclusive to the conventional systems (CONFYM and/or CONMIN).

There were few species with high relative abundance in all of the farming systems, which corresponded to weeds producing large numbers of small seeds with high persistence in the soil seedbank and/or with high dispersal ability, such as birdseye pearlwort (*Sagina procumbens* L.) (Dölle and Schmidt 2009). Some other species had high relative densities in the nonfertilized system, such as dwarf snapdragon [*Chaenorhinum minus* subsp. *minus* (L.) Lange in Willk. & Lange], whereas other species showed lower relative densities in the nonfertilized system than in the other systems. These species responded to fertilization, so the less relatively abundant species in NOFERT tended to be more nitrophilous species, such as manyseed goosefoot (*Chenopodium polyspermum* L.) (Ellenberg 1991). Many of these nitrophilous species were common and abundant in conventional systems, which also favored species that were either resistant to or difficult to control with herbicides (Robinson and Sutherland 2002), such as blackgrass (*Alopecurus myosuroides* Huds.), manyseed goosefoot, and annual bluegrass (*Poa annua* L.). There were several species that were particularly abundant under bioorganic and biodynamic management, such as rockcress [*Arabidopsis thaliana* (L.) Heynh. in Holl et Heynh.], toad rush (*Juncus bufonius* L.), Persian speedwell (*Veronica persica* Poir.), and thymeleaf speedwell (*Veronica serpyllifolia* L.).

**Seedbank Abundance and Diversity.** We found that 30 yr of contrasted farming practices had a significant influence on the weed seedbank size and diversity (Figure 1), as previously demonstrated (Albrecht 2003; Ryan et al. 2010). The seedbank size of the NOFERT system was significantly higher compared with the fertilized systems (Table 1), which was reported in other studies (De Cauwer et al. 2010). Seedbank density was also significantly higher in organic (BIOORG and BIODYN) compared with conventional (CONFYM and CONMIN) farming systems (Table 1). This pattern was also found by Ryan et al. (2010).

Higher nitrogen availability tends to promote crop growth, because crops are usually better competitors than most arable weeds. In this situation most weeds are at disadvantage for light and nutrient uptake, which may negatively affect seed production and, consequently, the replenishment of their seeds in the soil seedbank (Andreasen et al. 2006; Moonen and Barberi 2004; Pyšek and Lepš 1991).

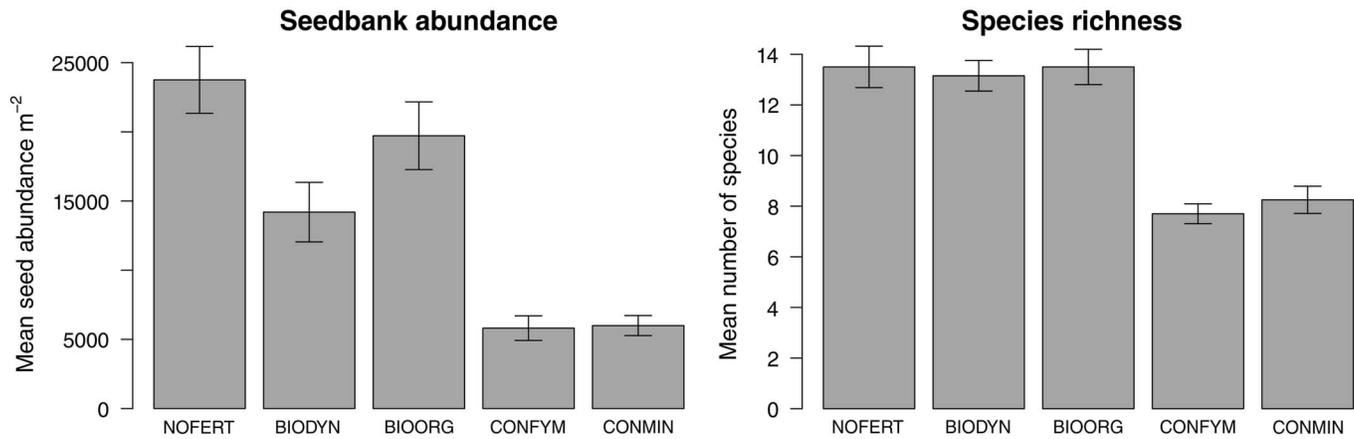


Figure 1. Mean ( $\pm$  SE) seed abundance (number of seeds  $m^{-2}$ ) and mean ( $\pm$  SE) species richness of weed species in the soil seedbank samples of the different farming systems: unfertilized (NOFERT), biodynamic (BIODYN), organic (BIOORG), mineral (CONMIN), and conventional (CONFYM).

This, together with the herbicide use, would partly explain why farming systems with higher nitrogen availability (CONFYM and CONMIN) had lower weed seed density in the soil seedbank. However, the availability of nitrogen is not only dependent on the amount of fertilizer but also on the form in which the fertilizer is delivered. Mineral fertilization and organic amendments differ in their nutrient release, with the latter having slower rates of nutrient release. Different nutrient release of fertilizers has been reported as an important factor that influences weed seedbanks (De Cauwer et al. 2010), and it may have affected the soil seedbank of the DOK long-term trial.

The reduced seedbank size of the CONFYM and CONMIN farming systems could be explained by the herbicide applications. Herbicides reduce weed populations and, consequently, inputs to the weed seedbank, because they limit both weed growth and seed production (Bàrberi et al. 1998; José-María and Sans 2011; Menalled et al. 2001; Potts et al. 2010).

A similar pattern was found for species richness, with higher values in the NOFERT system with respect to the other fertilized systems and also higher species richness in organic (BIOORG and BIODYN) with respect to the conventional (CONFYM and CONMIN) farming systems (Table 1). The increase in species richness in the seedbank under low nitrogen inputs and/or organic farming has also been reported by other authors (e.g., Albrecht 2005; Armengot et al. 2011; Boguzas et al. 2004; Ryan et al. 2010; Squire et al. 2000). This may have been due to the direct suppression of weed populations by herbicide applications in conventional systems (José-María and Sans 2011;

Menalled et al. 2001) and also indirectly through higher crop competition with weeds because of the mineral fertilization (Moonen and Bàrberi 2004; Pyšek and Lepš 1991).

**Seedbank Species Composition.** Farming systems not only had a significant impact on the size and diversity of the weed seedbank but also on its composition, as reported in other studies (e.g., Menalled et al. 2001; Ryan et al. 2010). The NMDS analysis based on presence/absence data ( $k = 2$ , nonmetric

Table 1. Coefficients and SEs of the linear mixed-effect models testing the effect of the farming system on the seedling abundance (A) and species richness (B) of the soil seedbank.

Contrasts <sup>a</sup>	Estimate $\pm$ SE	df <sup>b</sup>	
<b>A. Seedling abundance</b>			
Intercept	9.196 $\pm$ 0.121	4	***
NOFERT vs. Fert	0.178 $\pm$ 0.042	16	***
Org vs. Con	0.52 $\pm$ 0.094	16	***
BIODYN vs. BIOORG	-0.193 $\pm$ 0.133	16	
CONFYM vs. CONMIN	-0.043 $\pm$ 0.133	16	
<b>B. Species richness</b>			
Intercept	11.22 $\pm$ 0.517	4	***
NOFERT vs. Fert	0.57 $\pm$ 0.152	16	**
Org vs. Con	2.675 $\pm$ 0.341	16	***
BIODYN vs. BIOORG	-0.175 $\pm$ 0.482	16	
CONFYM vs. CONMIN	-0.275 $\pm$ 0.482	16	

<sup>a</sup> Orthogonal contrasts to compare the different levels of the farming systems: conventional (CONFYM), mineral (CONMIN), organic (BIOORG), biodynamic (BIODYN), and unfertilized (NOFERT). Groups of farming systems were: fertilized systems (Fert, CONFYM, CONMIN, BIOORG, and BIODYN), organic systems (Org, BIOORG and BIODYN), and conventional systems (Con, CONFYM and CONMIN).

<sup>b</sup> Degrees of freedom (df); statistical significance: \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

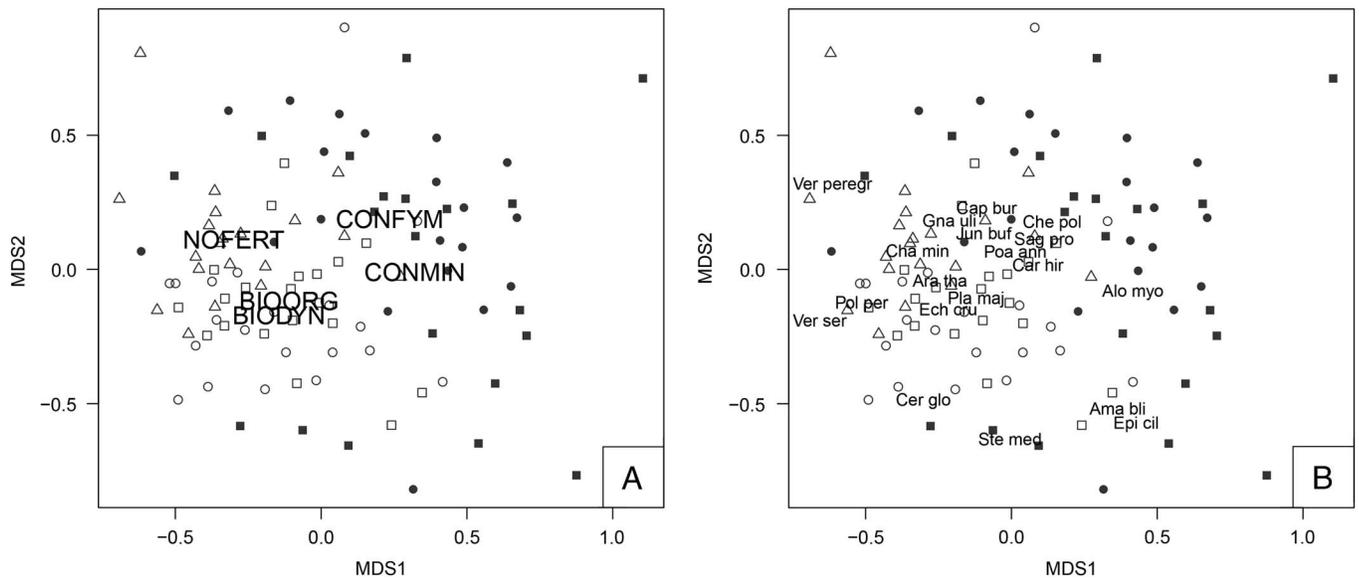


Figure 2. Site ordination (NMDS) based on floristic similarities of the different farming systems: conventional (CONFYM, black circles), mineral (CONMIN, black squares), organic (BIOORG, white squares), biodynamic (BIODYN, white circles), and unfertilized (NOFERT, white triangles) of 200 soil seedbank samples ( $k = 2$ , nonmetric fit = 0.928). (A) The labels of each treatment are cited on the average obtained after fitting the factor onto the ordination ( $r^2 = 0.2727$  and  $P = 0.0001$ ). (B) The labels of the most present species were fit onto the ordination: Alo myo: *Alopecurus myosuroides* Huds.; Ama bli: *Amaranthus blitoides* S. Watson; Ara tha: *Arabidopsis thaliana* (L.) Heynh. in Holl et Heynh.; Cap bur: *Capsella bursa-pastoris* (L.) Medik.; Car hir: *Cardamine hirsuta* L.; Cer glo: *Cerastium glomeratum* Thuill.; Cha min: *Chaenorhinum minus* subsp. *minus* (L.) Lange in Willk. & Lange; Che pol: *Chenopodium polyspermum* L.; Ech cru: *Echinochloa crus-galli* (L.) Beauv.; Epi cil: *Epilobium ciliatum* Raf.; Gna uli: *Gnaphalium uliginosum* L.; Jun buf: *Juncus bufonius* L.; Pla maj: *Plantago major* L.; Poa ann: *Poa annua* L.; Pol per: *Polygonum persicaria* L.; Sag pro: *Sagina procumbens* L.; Ste med: *Stellaria media* (L.) Vill.; Ver peregr: *Veronica peregrina* subsp. *peregrina* L.; and Ver ser: *Veronica serpyllifolia* L.

fit:  $r^2 = 0.928$ ) showed a clear spread of the soil samples according to the farming systems (Figure 2A). It particularly separated the weed communities by the NOFERT, organic systems (BIOORG and BIODYN), and conventional systems (CONFYM and CONMIN). The weed species composition of the BIOORG and BIODYN systems was more similar to the NOFERT system than to that of the conventional systems (Figure 2A). The fit of the farming system onto the plot was statistically significant according to the permutation multivariate ANOVA ( $r^2 = 0.2717$ ,  $P = 0.0001$ ), which revealed that farming systems determined the floristic composition of the soil seedbank. Therefore, the type and the intensity of management, mainly through the amount and the nature of fertilization and the type and intensity of weed control, defined the composition of the species in the soil seedbank (Ryan et al. 2010; Squire et al. 2000), and it reflected the long-term cumulative effects of agricultural management (Hawes et al. 2010).

For instance, there were certain species appearing mainly in the NOFERT system, such as dwarf snapdragon, prostrate knotweed (*Polygonum aviculare* L.), and ladysthumb (*Polygonum*

*persicaria* L.) (Figure 2B), that probably responded to the lower fertilization levels. The BIODYN, BIOORG, and NOFERT systems were separated from the CONMIN and CONFYM soil samples in the NMDS ordination because of the higher presence of ladysthumb, thymeleaf speedwell, sticky chickweed (*Cerastium glomeratum* Thuill.), and common chickweed [*Stellaria media* (L.) Vill.], among others (Figure 2B). These species could be favored in the organic farming systems by the nonuse of herbicides and by the lower crop competition due to organic fertilization (Andreasen et al. 2006; Pyšek and Lepš 1991). The CONFYM and CONMIN farming systems had blackgrass, manyseed goosefoot, bitter hairycreed (*Cardamine hirsuta* L.), and shepherd's-purse [*Capsella bursa-pastoris* (L.) Medik.] as the main characteristic species, which indicated that these species might be less affected by chemical weed control or more related to soils with higher nitrogen availability. In fact, most of these species are considered nitrophilous species by Ellenberg (1991).

**Specific Management Filters in the Community Assemblage.** Fertilization and herbicide applications acted as filters in assembling the arable weed

Table 2. Coefficients and SEs of the linear mixed-effect models testing the effect of the farming system on the weed community—weighted average Ellenberg indicator value and on the relative abundance of herbicide-resistant species.

Contrasts <sup>a</sup>	Ellenberg indicator value			Herbicide resistance		
	Estimate ± SE	df <sup>b</sup>		Estimate ± SE	df <sup>b</sup>	
Intercept	6.173 ± 0.075	4	***	0.351 ± 0.031	4	***
NOFERT vs. Fert	-0.086 ± 0.037	16	**	-0.025 ± 0.013	16	*
Org vs. Con	-0.169 ± 0.083	16		-0.069 ± 0.029	16	**
BIODYN vs. BIOORG	0.120 ± 0.117	16		0.009 ± 0.042	16	
CONFYM vs. CONMIN	-0.031 ± 0.117	16		-0.020 ± 0.042	16	

<sup>a</sup> Orthogonal contrasts were used to compare the different levels of the factor farming system: conventional (CONFYM), mineral (CONMIN), organic (BIOORG), biodynamic (BIODYN), and unfertilized (NOFERT). Groups of farming systems were: fertilized systems (Fert, CONFYM, CONMIN, BIOORG, and BIODYN), organic systems (Org, BIOORG and BIODYN), and conventional systems (Con, CONFYM and CONMIN).

<sup>b</sup> Degrees of freedom (df); statistical significance: \*,  $P < 0.1$ ; \*\*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ .

communities of the soil seedbank. The differences in the levels of the soil ammonia and nitrate supply may have selected different arable species, as weed species can vary in their affinity to nutrient availability (Ryan et al. 2010). We found a significantly lower weighted average nitrogen Ellenberg indicator value in weed communities of the NOFERT system compared with the four fertilized systems (Table 2). This result confirmed the role of nitrogen availability as a filter assembling the weed communities of the soil seedbank, as previously reported (Ryan et al. 2010). We also found a significant increase in the relative abundance of weeds with higher nitrogen Ellenberg indicator values under conventional systems (CONFYM and CONIN) compared with the organic systems. Conventional systems received higher fertilizer inputs than the organic ones (Mäder et al. 2006), but these fertilizers also differed in their forms and compositions; therefore, nutrient availability and time to release were other factors influencing the weed community composition of the seedbanks (De Cauwer et al. 2010). We found that organic farming systems and the NOFERT system involved less abundance of nitrophilous species than conventional systems, which was in accordance with previous studies (Hawes et al. 2010). Hence, even though conventional management reduced the weed seedbank density, at the same time, it favored community assemblies with more nitrophilous species, which are highly competitive and could strongly outcompete the crop (Fried et al. 2009; Moonen and Bàrberi 2004).

Herbicide applications promote resistant species (Derksen et al. 2002). Accordingly, we found that the long-term application of herbicides led to weed communities with higher abundances of

herbicide-resistant species (Table 2) compared with weed communities found under the farming systems without herbicide applications. We may ascertain that herbicide resistance would be developed under conventional farming systems (CONFYM and CONMIN); therefore, the most susceptible species to herbicides would be eradicated from these systems. In this sense, herbicide application acts as a community filter by selecting against susceptible weed species and promoting the ones that are resistant to herbicides. In contrast, organic farming systems would preserve those species that are susceptible to herbicide applications (Hawes et al. 2010; Ryan et al. 2010).

**Implications for Conservation.** This study showed that different long-term farm management systems had significant influences on the size, species richness, and composition of the weed seedbank. Seed abundance and species richness were higher in organic and biodynamic farming systems than in conventional and mineral farming systems. This trend may be attributable to organic fertilization, which reduced the competitive pressure exerted by the crop because of the lower amount of nutrients applied and their slower release. Moreover, mechanical weed control is less intensive than herbicide applications to control weeds. The different farming systems determined shifts in species assemblages by acting as filters selecting against certain species but promoting others. Specifically, high inputs of mineral fertilizers selected for more nitrophilous species, while herbicide applications selected against herbicide-susceptible species.

The promotion of weed species diversity should be an objective in agricultural policy, given the

significant decrease in these species in recent decades and the notable role of weed species diversity in ecosystem functioning and in the characterization of agricultural landscape. The conversion to organic or biodynamic farming practices, with lower fertilizer inputs and without herbicide applications, would support weed communities. However, minimizing fertilizer inputs, especially mineral fertilizers, and avoiding herbicide applications may not be accepted well among farmers, because despite improving species richness, it also increases weed seed density, creating the potential for future infestations. Therefore, improvements in weed control strategies should be considered to control weed seed density in the soil seedbanks of organic farming systems while promoting species diversity and maintaining the species composition to avoid losing more susceptible species to agricultural intensification.

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