



Research Article

Volume 23 Issue 4 - January 2020

DOI: 10.19080/ARTOAJ.2020.23.556244

Agri Res & Tech: Open Access J

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Organic Cropping Systems do not Increase Weed Seed Numbers but do Increase Weed Diversity



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Submission: January 10, 2020 ; **Published:** January 17, 2020

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Abstract

The influence of different cropping systems on the soil weed seed bank after the first crop rotation within a five-field crop rotation (barley undersown with red clover, red clover, winter wheat, pea, potato) in three organic (Org) and in two conventional (Conv) cropping systems was investigated. In organic systems Org I and Org II cover crops were incorporated as a source of nutrient inputs to the soil and in Org II composted cattle manure was also applied. The Org 0 acted as the organic control system without cover crops and manure. The two conventional cropping systems were treated with herbicides and fungicides and differed in fertilizer application (i.e. Conv I no fertilizer use (as control) and Conv II mineral fertilizer use). In general, the lowest number of annual weed seeds was found in system Conv I, the highest in Conv II. In organic systems with cover crops (Org I, II) there was a strong tendency for decreased weed seed numbers and increased biodiversity. The highest values of the Shannon-Wiener diversity index and Margalef richness index were in Org II system. In all systems the most abundant species in weed seed banks were *Chenopodium album* L. and *Viola arvensis* Murr.

Keywords: Equitability index; Margalef index; Shannon-Wiener diversity index; Weed seed bank; Winter cover crops

Introduction

In organic agriculture one of the main challenges is to acquire extensive review of the biological and ecological behaviour of weeds for effective weed control [1,2]. To overcome annual weed infestation, it is important to define the dynamics of weediness, which has been acknowledged to greatly depend on the quantity and distribution of the soil seed bank [3,4]. Soil seed banks are viable seed stocks in the soil and on its surface [5], accumulating or remaining until germination [6]. Seed banks consist of seeds, which may be produced over several years or emerged recently [5,7]. Both seed banks and aboveground vegetation in agroecosystems are very variable – seed banks due to emergence in seasonal patterns during the vegetation year [8] and aboveground vegetation is greatly affected by management during a given year and competition with the crop [2]. Correct evaluation of the composition of the weed seed bank reflects past field management and may make it possible to predict weed problems of the future [1,2].

Organic and conventional management systems address problems differently. Conventional farming depends mainly on

short-term solutions e.g. herbicide and fertilizer application, which in turn affect the weed seed bank [9-11] and reduce species diversity [1,2,12]. Organic systems on the other hand rely on longer-term preventative solutions. Recognition of the importance of the nutrient cycling and weed, pest and disease control are essential either way in agronomy [11,13]. Successful weed seed management strategies include decreasing the germinable fraction of the seed bank, thereby manipulating the emergence and germination of weeds [4,14]. Also, for effective aboveground weed control it is necessary to limit weed seed production of already emerged plants and to stimulate seed death to reduce the seed bank size [15,16].

Using agricultural practices for changing the seed bank results in changes in weed flora [16,17]. The main practices which have a sufficiently severe impact on weed flora and thus on the weed seed bank are crop rotational sequences and tillage [17]. As a part of the rotational crop sequences, winter cover crops are introduced in the crop rotation with spring sown crops [18–22]. The content of organic carbon in the soil is improved if winter cover crops are

incorporated in the soil [23], benefitting microbial activity [24]. The seeds in the soil are subject to microbial decomposition by soil biota [4] thereby enriching the soil and helping to reduce weed problems in the field [22]. Cover crops have an effect on the establishment of weeds and thereby contribute to reduction of weed seed formation and accumulation of seeds in the soil [18].

Weed population dynamics as well as species composition are influenced by seed dynamics within the soil during farming practices [25]. The important role of seed in the weed life cycle

as the origin of future populations [6] has been recognised. Therefore, the present study assesses the effects of different cropping systems with the same rotational schemes on soil weed seed bank and its changes after one 5-year crop rotation. The aim of the study was to investigate the influence of conventional and organic cropping systems on the weed seed bank. In organic systems winter cover crops were used. Therefore, the hypothesis was that winter cover crops regulate weed seed occurrence to a comparable level as herbicides in conventional systems.

Materials and Methods

Table 1: The treatments in the organic and conventional farming systems.

Crop rotation	Organic Systems			Conventional Systems	
	Org 0 – control (Crop rotation)	Org I (Crop rotation + green manure as winter cover crops) Winter oilseed turnip + winter rye	Org II (Crop rotation + green manure + composted cattle manure)	Conv I – control (Crop rotation + herbicides + fungicides + insecticides)	Conv II (Crop rotation + herbicides + fungicides + insecticides + mineral fertilizers)
Winter wheat		Winter oilseed turnip + winter rye	10 t ha ⁻¹		25 kg ha ⁻¹ P, 95 kg ha ⁻¹ K; 150 kg ha ⁻¹ N
Pea		Winter oilseed turnip			25 kg ha ⁻¹ P and 95 kg ha ⁻¹ K; 20 kg ha ⁻¹ N
Potato		Winter rye	20 t ha ⁻¹		25 kg ha ⁻¹ P and 95 kg ha ⁻¹ K; 150 kg ha ⁻¹ N
Barley us. red clover			10 t ha ⁻¹		25 kg ha ⁻¹ P and 95 kg ha ⁻¹ K; 120 kg ha ⁻¹ N
Red clover		Red clover			

The five-field crop experiment with three different organic and two conventional systems was set up in 2008 and located at the test site of the Estonian University of Life Sciences in Eerika (58°22' N, 26°40' E). The soil type of the experiment area was sandy loam Stagnic Luvisol according to the World Reference Base classification [26]. The mean characteristics of the humus horizon were as follows: Corg 1.1-1.2%, Ntot 0.10-0.12%, P 110-120 mg kg⁻¹, K 253-260 mg kg⁻¹, pHKCl 5.9, soil bulk density 1.45–1.50 g cm⁻³. The crops grown in succession were as follows: barley (*Hordeum vulgare L.*) undersown with red clover (*Trifolium pratense L.*), red clover RC (*Trifolium pratense L.*), winter wheat (*Triticum aestivum L.*), pea (*Pisum sativum L.*) and potato (*Solanum tuberosum L.*). The experiment was established in 5 cropping systems (Org 0, Org I, Org II, Conv I and Conv II) with 5 crops in four replications (each plot 60 m²) situated in a systematic block design (100 plots in total); (Table 1) [24]. Organic (Org) and conventional (Conv) plots were separated by an 18 m long section of mixed grasses to avoid contamination with synthetic pesticides, mineral fertilisers and winter cover crops.

System Org 0 followed the above-mentioned rotation. In system Org I, winter cover crops as green manure was used: mixture of winter rye (*Secale cereale L.*) and winter oilseed turnip (*Brassica rapa L. var. oleifera*) after winter wheat, winter oilseed turnip after pea and winter rye after potato. In system Org

II, in addition to winter cover crops, composted cattle manure was applied at the rate of 20 t ha⁻¹ for potato, and 10 t ha⁻¹ for winter wheat and barley [22]. Red clover was mown twice – in the middle of June and in the second half of August. Cover crops were sown immediately after harvest. However, prior to sowing the subsequent crop, all cover crops were incorporated into the soil as green manure. The organic systems were ploughed in spring (at the beginning of May), conventional systems in autumn (at the end of October).

Besides organic systems there were two conventional farming systems without winter cover crops: Conv I (no fertilizer use) and Conv II (all the crops received 25 kg ha⁻¹ P and 95 kg ha⁻¹ K; also winter wheat and potato 150 kg ha⁻¹ N, barley undersown with red clover 120 kg ha⁻¹ N and pea 20 kg ha⁻¹ N). Both conventional systems were treated with herbicides, insecticides and fungicides. Plots with red clover did not receive any mineral fertilizers or chemical pest control [27].

The weed seed bank samples were taken in 2015 with soil borers (15 mm diameter) after crop harvest and before autumn ploughing. From each plot 16 soil samples were taken from the depth of 0-25 cm soil layer. Samples of each plot were mixed together in a bucket. Seeds were extracted from a 500 g portion of the soil sample using a flotation-based method [28]. Weed

seeds were separated from the soil by potassium carbonate (K₂CO₃) aqueous solution. For preparation of the solution 2.0 kg of potassium carbonate were dissolved in 1.8 l of water. Weed seeds were counted and species identified under the microscope.

The number of weed seeds in the seed bank was calculated to an area of 1 m² of plot area using the formula Eq. 1 [29,30] as shown below:

$$N = (h * \rho * n * 10) / Wd$$

where: N – number of viable seeds (n m⁻²); h – depth of plough layer (cm); ρ – soil bulk density (g cm⁻³); n – counted number of seeds in the soil sample; Wd – weight of dry soil sample (g).

The number of seeds and the species composition of weed seed communities were used to assess the diversity.

Species diversity indexes are aimed to unite information on the relative abundance and richness of species into a single value, varying on how much accent one of these two components are bearing [31–33]. The Shannon-Wiener is one of the oldest and most commonly used diversity indexes [34]. If Shannon-Wiener index is for taking account of how individuals are distributed within the species, then species richness (Margalef) index shows the total number of species present in a given sample or area [35].

The diversity indexes (Eq. 2) of weed seed species were calculated as Shannon-Wiener index [36,37] of weed seed species diversity H':

$$H' = -\sum_{i=1}^S pi \ln(pi) \quad (2)$$

The species richness measure was calculated as Margalef index (d), Eq 3 [38]:

$$d = \frac{s-1}{\ln N} \quad (3)$$

The Equitability index is the ratio of the population diversity and is affected by the relative quantity of species in a community

[39]. It could be calculated when the variation between populations is missing [40] and is usually on a scale from 0 (shows high dominance by one species or low evenness) to 1 (indicates even abundance of all species or ultimate evenness) [41,42]. The formula to calculate Equitability index, Eq 4 [43,44] was as follows:

$$J' = \frac{H'}{H'_{max}} \quad (4)$$

Statistical analyses were performed using the Statistica software package (version 12.0). Significant differences between cropping systems were tested by Fisher's least significant difference test. The statistical significance level was set at p=0.05. Full-factorial analysis of variance (ANOVA) was used to test the statistical significance of crop and cropping systems and their interaction effects on summer and winter annual weed seed species. The normality of the data was assessed by the Kolmogorov-Smirnov test.

Results and Discussion

The number of seeds in the soil changes in time, but presumably seed production is related to aboveground density or biomass of weeds [45]. Seed bank size is also affected by different factors, such as annual in situ seed production, addition by wind-dispersed seeds, seed rain [1], contamination by machinery and manure [46]. The current study focuses on seed bank description depending on different treatments in fertilizing and cultivation.

In 2015 a total of 23 weed species were found. The most dominant species were *Chenopodium album* L. among summer annuals and *Viola arvensis* among winter annuals. The number of winter annual seeds was about 25 times less than summer annual seeds (Table 2). In 2011 [27] the same species were also dominant but the number of seeds was lower. The number of weed seeds was dependent on cropping systems as well as on the crop grown (Table 2) (Figure 1).

Table 2: Number of annual (summer, winter and total) weed seeds in the soil of crop rotation in 2015.

Crop	Weed seeds	Number of seeds, 1000 seeds per m2				
		Conv I	Conv II	Org 0	Org I	Org II
Red clover	Total summer annual	25.34a	36.19ab	43.26b	35.77ab	28.70ab
	<i>Chenopodium album</i>	23.24a	32.48ab	40.04b	32.06ab	25.55a
	Total winter annual	1.12a	2.87ab	2.24ab	3.08ab	4.90b
	<i>Viola arvensis</i>	0.84a	2.38ab	1.33ab	1.68ab	3.29b
	Total	26.46a	39.06ab	45.50b	38.85ab	33.60ab
Winter wheat	Total summer annual	33.04a	30.17a	31.64a	28.14a	27.86a
	<i>Chenopodium album</i>	30.87a	27.58a	27.37a	25.27a	24.78a
	Total winter annual	2.87a	5.95a	3.78a	4.06a	4.06a
	<i>Viola arvensis</i>	2.38a	5.18a	1.75a	2.38a	1.96a
	Total	35.91a	36.12a	35.42a	32.20a	31.92a

Pea	Total summer annual	35.63ab	45.08b	27.30a	35.21ab	38.36ab
	Chenopodium album	32.41ab	41.86b	25.27a	32.13ab	33.53ab
	Total winter annual	3.43a	4.34a	3.08a	3.99a	3.71a
	Viola arvensis	2.87ab	4.20b	1.40a	1.61a	1.40a
	Total	39.06ab	49.42b	30.38a	39.20ab	42.07ab
Potato	Total summer annual	27.43a	33.02a	34.00a	30.29a	27.76a
	Chenopodium album	24.51a	31.14a	31.92a	26.98a	25.09a
	Total winter annual	1.37a	3.77b	3.06ab	2.15ab	3.25ab
	Viola arvensis	1.17a	2.67a	1.69a	1.17a	1.82a
	Total	28.80a	36.79a	37.05a	32.44a	31.01a
Barley, us. red clover	Total summer annual	34.23a	39.97a	34.93a	39.06a	37.10a
	Chenopodium album	31.29a	36.19a	32.27a	35.98a	34.86a
	Total winter annual	1.96a	3.29a	2.73a	1.96a	3.99a
	Viola arvensis	1.61ab	3.01b	1.89ab	1.05a	1.82ab
	Total	36.19a	43.26a	37.66a	41.02a	41.09a

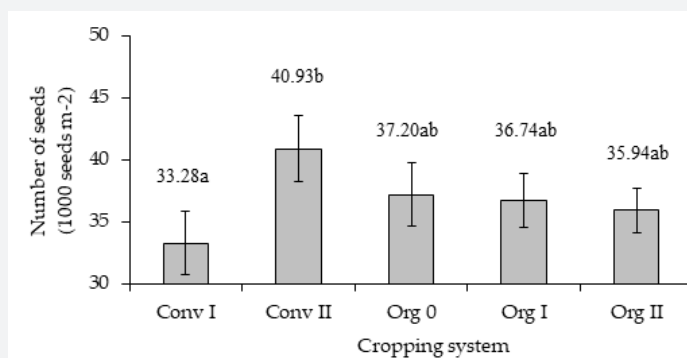


Figure 1: Total number of weed seeds, 1000 seeds per m² in the soil of crop rotation crops in 2015 (ANOVA, Fisher (LSD) test). Means followed by different letters within each bar indicate significant differences ($p < 0.05$) within cropping systems; 2 – Vertical bars denote the standard errors

The seed bank of annual species varied from a minimum of 26,460 seeds m⁻² in red clover in Conv I to a maximum of 49,420 seeds m⁻² in pea in Conv II.

As an average of all cropping systems the highest seed number was found in Conv II compared to Conv I and all organic systems (Figure 1). It shows that the use of herbicides in systems Conv I and Conv II did not control the occurrence of seeds. In Conv II the highest seed number could be explained by the influence of mineral fertilizers on weed seed production. In all organic systems the number of seeds was lower than in Conv II and the lowest amount of seeds was found in system Org II where composted manure was used. System Org II had also the lowest number of seeds in 2011 [27]. Although manure in system Org II could be a source of weed seeds [46], it can also stimulate the activity of microorganisms and increase enzyme activity in soil [24]. The trend of larger weed seed number in system Org 0 was probably due to the lack of winter cover crops. As Dorn et al. [47] has stated,

cover crops either growing or incorporated into the soil have an inhibitory effect on weeds.

Cover crops, when incorporated in a cropping system, have numerous beneficial effects: better weed suppression ability, increased soil microbiotic activity and abundance of seed predators, which can affect the abundance and distribution of weed seeds [48].

Compared to summer annual seed numbers, the percentage of winter annual was much lower (Table 2). The highest number of *C. album* seeds was found in the conventional system (Conv II) in the pea crop, although Barberi et al. [1] have stated that the population size of *C. album* did not depend on the management system. The effect in pea most likely occurred because the herbicide used did not have a comprehensive impact. In addition, eliminating some species may cause species impoverishment in general biodiversity [49] and will allow species such as *C. album* to establish a stable

seed bank and thus the ability to maintain viability in the soil [6]. As shown in (Table 3), the main factor which influenced winter

annual weed seeds (including *V. arvensis*) was the cropping system.

Table 3: The effects of different factors on summer and winter annual weed seed species in 2015.

Factor	Summer annual	incl. <i>Chenopodium album</i>	Winter annual	incl. <i>Viola arvensis</i>	Total
Crop (C)	F4.75 = 2.051, p = 0.096	F4.75 = 1.988, p = 0.105	F4.75 = 2.062, p = 0.094	F4.75 = 1.266, p = 0.291	F4.75 = 1.722, p = 0.154
Cropping system (CS)	F4.75 = 1.005, p = 0.411	F4.75 = 1.021, p = 0.402	F4.75 = 3.067, p = 0.021*	F4.75 = 4.722, p = 0.002*	F4.75 = 1.349, p = 0.260
C x CS	F16.75 = 0.858, p = 0.618	F16.75 = 0.823, p = 0.656	F16.75 = 0.484, p = 0.948	F16.75 = 0.774, p = 0.710	F16.75 = 0.756, p = 0.728

In red clover the number of summer annual seeds as well as the total seed count was statistically higher in system Org 0, with the lowest in system Conv I (Table 2). Both of the systems acted as control systems; however, the difference was remarkable mainly due to the use of herbicides in system Conv I. Ball and Miller [50] have reported that herbicides can influence seed bank composition, with some of the weed species decreasing and some increasing. Previous studies found that the composition of weeds and the weed seed communities were influenced by mowing the red clover [40]. The number of *C. album* was statistically higher in system Org 0. The increased weed seed amount may be a result of the previously sown barley's greater dependence on the growing conditions, especially weather and fertilization [22]. The results of our study showed that the weed seed number in the following winter wheat was lower than in the previous red clover.

In pea all of the peaks and lows appeared within systems Conv II and Org 0 (Table 2). The number of weed seeds of summer annual species was significantly higher in Conv II than in any of the other management system. Org 0 on the other hand had a trend of all of the lowest counts of weed seeds compared to the other systems. The high weediness in pea has been mentioned earlier over the

course of the organic experiments on the same plots [22] and the reason for the high weed seed infestation in all of the systems (except for system Org 0 where no cover crops were grown) is the weak competitiveness of the pea, also the carry-over effect of the cover crops. This finding is inconsistent with De Cauwer et al. [4] who reported that the higher seed density occurred in plots where no mineral fertilizer was used.

In potato and barley undersown with red clover, the differences were not significant, but the least amount of weed seeds was found in system Conv I. Some researchers have noticed that when herbicides are applied continuously the total seed bank shows signs of declining [40,50,51]. In system Conv II the added mineral fertilizers may have had an effect on the weed seeds by increasing weed productivity [52]. Our data indicate that organic cropping systems, especially enriched by cover crops and compost, can regulate weed seed occurrence on an even better level than herbicides. Compared to the data of the initial study in 2011 [27] the dominant weed species, (*Chenopodium album* among summer annuals and *Viola arvensis* among winter annuals) remained unchanged during the 5-year crop rotation.

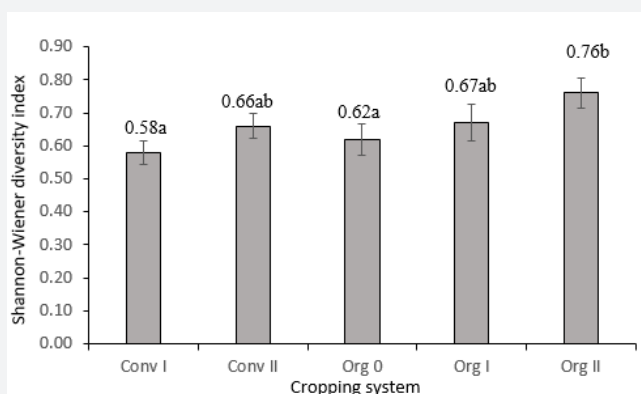


Figure 2: Shannon-Wiener diversity indexes in 2015 (ANOVA, Fisher (LSD) test). Means followed by different letters within each bar indicate significant differences ($p < 0.05$) cropping systems; Vertical bars denote the standard errors.

Table 4: The effects of different factors on Shannon-Wiener diversity index, Margalef richness index and Equitability index in 2015.

Factor	Shannon-Wiener diversity index, H'	Margalef richness index	Equitability index, J'
Crop (C)	F4.75 = 1,929, p = 0,114	F4.75 = 0,359, p = 0,837	F4.75 = 2,034, p = 0,098
Cropping system (CS)	F4.75 = 2.368, p = 0,060	F4.75 = 3,163, p = 0,019*	F4.75 = 1,395, p = 0,244
C x CS	F16.75 = 0,642, p = 0,839	F16.75 = 1,126, p = 0,348	F16.75 = 0,932, p = 0,981

As an average, Shannon-Wiener diversity index values (Figure 2) were higher in organic systems. The lowest indexes were calculated in systems where no winter cover crops were used – Conv I and Org 0 and highest S-W index was in Org II system. Major et al. [53] reported that addition of compost and inorganic fertilizer has an effect on soil fertility and thus an increase in weed species richness. Some authors [33,51,54] have shown, that the addition of mineral and/or organic fertilizer may reduce plant diversity by favouring competitive species, but this did not occur in the present study. The analysis of effects of different factors

on S-W index (Table 4) was made, but no significant conclusions could be made.

Margalef richness indexes were highest in organic systems with treatment (Org I, Org II); (Figure 3). The richness index was affected by field management, where control system (Org 0) without fertilizer decreased the index and winter cover crops and manure (Org II) increased it. This is also confirmed by Dölle and Wolfgang [55], who pointed out that species richness is greatly linked to yearly disturbance – the process of incorporating manure into the soil.

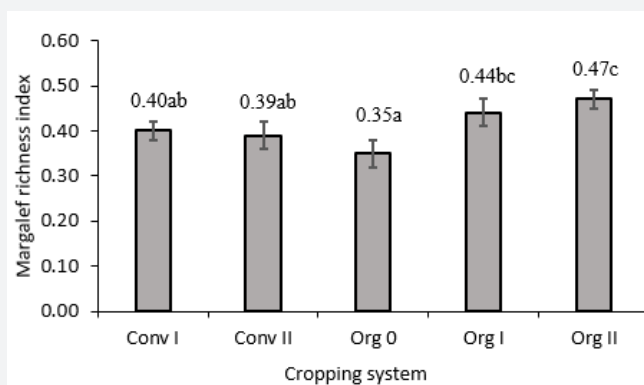


Figure 3: Margalef richness indexes in 2015 (ANOVA, Fisher (LSD) test). Means followed by different letters within each bar indicate significant differences ($p < 0.05$) in cropping systems; Vertical bars denote the standard errors.

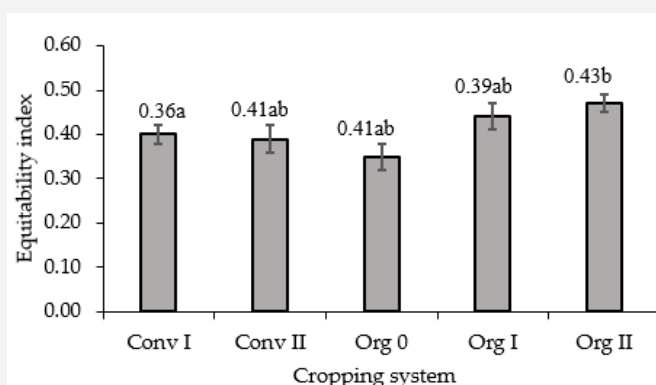


Figure 4: Equitability indexes in 2015 (ANOVA, Fisher (LSD) test). Means followed by different letters within each bar indicate significant differences ($p < 0.05$) in cropping systems; Vertical bars denote the standard errors.

All values of the Equitability indexes were under 0.5 (Figure 4), which indicates the unevenness of the weed species in the seed bank. In cropping systems, the statistical difference was seen in Org II and Conv I. This indicates that herbicide use does not affect the weed seeds of the different weed species equally in the soil seed bank. The lowest E index found in system Conv I (0.36), where herbicides were used, indicates that there were few dominant weed species in this system.

Conclusion

After one rotation a total of 23 weed species was found. The most abundant species in the weed seed bank were *Chenopodium album* and *Viola arvensis*. The highest number of *V. arvensis* as well as *C. album* seeds were found in conventional systems with the highest rate in pea (for *C. album*), where herbicides and fertilizers were used.

Differences in weed seed number between systems were inconsistent, but overall not higher in organic than conventional. Weed diversity tended to be a little higher in organic systems, which has potential environmental benefits. In general, all the organic systems showed tendencies of having the lowest seed numbers and highest Shannon-Wiener biodiversity and Equitability indexes. This tendency was clearest in system Org II, where winter cover crops were used in combination with composted manure. However, it is not clear from a system comparison which factor was affecting weeds – was it the fertilizer type, herbicides or cover crops? Such questions can be answered by future research, but from this study we conclude that organic systems have better weed management outcomes than conventional systems.

Acknowledgment

The study has been supported by ERA-NET Core organic project FertCrop, by Estonian University of Life Sciences projects 8-2/T13001PKTM, P170062PKTM and by Institutional Research Project IUT36-2. The deepest gratitude to I.H. Williams for English revision.

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DOI: [10.19080/ARTOAJ.2020.23.556244](https://doi.org/10.19080/ARTOAJ.2020.23.556244)

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