Research Article

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# Weed seed bank dynamics responses to long-term chemical control in a rice–wheat cropping system

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## Abstract

**BACKGROUND: It remains an open question if the long-term application of single chemical herbicides would inevitably lead to increased weed populations and result in out-of-control weeds. The annual dynamics of weed seed bank responses to different weeding measures (chemical herbicide, hand weeding and no weeding) in rice–wheat cropping systems were compared to observe the succession of weed communities under different weed selection pressures for 17 years.**

**RESULTS: In unweeded rice–wheat cropping plots, the initially dominant broadleaf weeds were overtaken by grasses and eventually by sedges, while in plots subjected to chemical herbicide or hand weeding, broadleaf weeds remained dominant followed by grasses. The rice–wheat cropping system favoured the spread of paddy weed species; weeding had little effect on the composition of the dominant rice weeds but greatly influenced that of wheat weeds. Total seed density tended to decrease in both weeded and unweeded plots, but the species density and composition of the seed banks differed among plots treated differently. Weeding slightly increased weed species diversity and decreased weed community evenness and dominance in the first several years, but this scenario could have negative consequences in the long term; however, without weeding, stronger interspecific competition led to a decrease in weed species diversity whereas weed community evenness and dominance increased.**

**CONCLUSION: Long-term and repeated application of pre-emergence chemical herbicides and hand weeding had similar effects on the weed community dynamics, indicating that exclusive application of pre-emergence herbicide could maintain the weed community at a durable relatively low infestation level.**


*Supporting information may be found in the online version of this article.*

**Keywords:** chemical herbicide; weed community succession; weed group variation; weed species composition; dominant weed species

## 1 INTRODUCTION

Weed management is closely related to weed community dynamics, and tracking the species, distribution and damage of weeds in crop fields and determining appropriate farming practices and weeding methods are broad concerns and valued. Changes in weed community composition are due to the selective pressure caused by changes in weed habitats under different farming practices.[1] Many farming practices, particularly soil improvement, such as fertilization and tillage practices,[2,3] rotation and continuous cropping and weeding methods[4,5] influence the composition, density and diversity of weed communities.[6] A soil weed seed bank demonstrates the existence of a weed community, a link of the growth phases of an ecosystem and a guarantee of weeds passing through the harsh environment and achieving population continuation.[7,8] Weed occurrence, growth and decline and succession in farmlands are closely related to the soil weed seed bank.[9] Research on the dynamics of seed bank populations in arable soils could be used to predict the infestation of weed populations and improve decision-making for managing specific weed problems.[10]

People have explored various approaches, techniques and methods for weed control in farmland. With labour savings, timeliness and normally high economic benefits, chemical herbicides are currently being widely used for weeding and will be an irreplaceable weeding tool for a long time. The long-term and extensive use of chemical herbicides has caused many problems, such as resistance, residue and pollution.[11] After years of using the same herbicide, a large number of herbicide-sensitive weed populations were killed and reduced, while other tolerant or resistant populations spread

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and increased, which led to a change in weed population and community structure, with the secondary species becoming the dominant species, increasing control difficulty.[12,13] The continuous application of butachlor, thiobencarb, and 2,4-D resulted in the predominance of perennial sedges, *Cyperus serotinus*, and *Eleocharis kurogawa* in paddy fields.[14] A previous study showed that the continuous or rotated application of pretilachlor or butachlor combined with 2,4-D caused the disappearance of *Hydrolea zeylanica* and the dominance of *Cynodon dactylon* in a rice–rape cropping system.[15] Due to the continuous use of butachlor and anilofos in paddy fields, the weed flora shifted to sedges, such as *Cyperus* sp., *Scirpus* sp., *Fimbristylis* sp., and *Eleocharis* sp., and broad-leaved weeds, such as *Caesulia axillaris*.[6] However, most reports on the long-term application of chemical herbicides leading to out-of-control weeds or resistant weeds are incomplete and lack long-term continuous observations of weed community succession under the long-term application of herbicides. At the same time, there are few studies focusing on the differences in long-term weed community dynamics between traditionally weeded (hand weeding) and unweeded areas under the same cropping system. A comparative study on weed community dynamics and succession with long-term chemical herbicide application, hand weeding and no weeding under the same cropping system is beneficial to determine the fundamental cause of weed population deterioration and weed resistance and explore reasonable weed management under this cropping system. Intensive cropping of winter wheat (*Triticum aestivum* L.) and summer rice (*Oryza sativa* L.) is the main cultivation pattern, which has lasted for several decades,[16] in the middle and lower reaches of the Yangzi River in China, and complex and stable weed distributions have occurred under this long-term single planting pattern. The objectives of this study are to compare the annual weed seed bank dynamics (variation in population composition and species density and biodiversity) under different weeding measures (chemical herbicide, hand weeding and no weeding) in a rice–wheat cropping system and provide a basis for exploring reasonable weed management.

## 2 MATERIALS AND METHODS

### 2.1 Experimental site

The experimental field was located on the farm of the Agricultural Science Institute along the Yangzi River of Jiangsu Province (31° 8′N, 120°38′E), Xueyao township, Nantong city, Jiangsu Province, China. The soil type was mucky soil with initial pH of 6.8, organic matter of 18.1 g kg$^{-1}$, total nitrogen of 1.24 g kg$^{-1}$, total phosphorus of 0.57 g kg$^{-1}$ and total potassium of 15.77 g kg$^{-1}$ in 2001. This study site located in the region which has a humid subtropical monsoon climate; the minimum, maximum and mean temperature and rainfall of the study area in wheat (from November to May of the following year) and rice (from June to October) cropping seasons from 2001 to 2017 are presented in Table 1.

### 2.2 Experimental design

The field experiment has been continually carried out since the wheat season of November 2000, with annual cropping of summer rice and winter wheat, involving four replicates of three treatments in a random block design. The following treatments were applied: (i) control (CK): no weeding; (ii) chemical herbicide (CH): in the wheat season, 1500 mL ha$^{-1}$ of acetochlor + isoproturon JG (Jiangsu Jialong Chemical Co., Ltd, Tongshan, China) was applied 2 or 3 weeks after wheat seeds were sown from 2001 to 2017, and in the rice season, 900 g ha$^{-1}$ of 53% mefenacet + bensulfuron methyl WP (Jiangsu Kuaida Agrochemical Co., Ltd, Matang, China) was applied 1 week after rice seedlings were transplanted from 2001 to 2014, which was changed to 720 g ha$^{-1}$ of 53% butachlor + bensulfuron methyl (Jiangsu Kuaida Agrochemical Co., Ltd) from 2014 to 2017; and (iii) hand weeding (HW): weeds were uprooted and removed two times at early tillering stage and 3 weeks later in each crop season by hand weeding. The plot of each treatment measured 15 m$^2$ (3 m × 5 m) and was isolated by cement ridges to prevent the interflow of water. More than 50% of weed seeds in paddy fields can float and be dispersed by irrigation water.[17,18] Therefore, to maintain the relative independent cycling of the weed seed bank in each plot, the intake and outlet of each plot were blocked with 0.125 mm of screen to prevent the dispersal of weed seeds with irrigation water. During irrigation, seeds of *Beckmannia syzigachne*, *Myosoton aquaticum*, *Monochoria vaginalis* and *Echinochloa crus-galli* were the most easily observed being blocked by the screens. Rice (Wuyoujing) seedlings were transplanted by hand in summer with a density of 30 plants·m$^{-2}$ (3 × 10$^5$ plants ha). Wheat (Yangmai No. 15) seeds were broadcast by hand in winter with a density of 1.5 kg m$^{-2}$(1.5 × 10$^4$ kg ha). Before transplanting and broadcasting, the soil of each plot was manually ploughed at a depth of 15 cm with spade and rake.

### 2.3 Seed bank sampling

Twelve soil cores that were 3.5 cm in diameter and 15 cm deep, which is equal to the plough layer, were equidistantly sampled from each treatment in late October after each rice harvest from 2001 to 2017. Samples from each individual treatment were smashed and air-dried in the net house. Subsequently, the soil samples were passed through a 4 mm sieve to remove large debris and stones. After mixin© 2019 Society of Chemical Industryg, each sample was divided into six parts (seed content of each part equalled seed density of 0.002 m$^2$ in 15 cm deep soil), three of which were determined by elutriation.[19] After elutriation, the remnant of each divided part was air-dried and put under a binocular dissecting mirror (the maximum magnification is 10 × 4 times) to determine the weed species and number of each species.

### 2.4 Data processing and analysis

The weed soil seed bank data from 2001 to 2017 were expressed as the number of weed seeds per square metre. Phytosociological structure was assessed by absolute and relative values of frequency, density, abundance and importance value for each species, which were computed by the following equations:[20]

$$\text{Absolute frequency}\,(AF)=\frac{\text{number of plots with species present}}{\text{total number of plots}}$$

$$\text{Relative frequency}\,(RF)=\frac{\text{species absolute frequency}}{\text{sum of all absolute frequency}}\times100$$

$$\text{Absolute density}\,(AD)=\frac{\text{total number of seeds of a species}}{\text{total sampled area}}$$

$$\text{Relative density}\,(RD)=\frac{\text{species absolute density}}{\text{sum of all absolute density}}\times100$$

$$\text{Absolute abundance}\,(AA)=\frac{\text{total number of seeds of a species}}{\text{total number of plots containing that species}}$$

$$\text{Relative abundance}\,(RA)=\frac{\text{species absolute abundance}}{\text{sum of all absolute abundance}}\times100$$

$$\text{Important value}\,(IV)=RF+RD+RA$$



  

**Table 1.** The minimum ($T_{min}$), maximum ($T_{max}$) and mean ($T_{mean}$) temperature and rainfall of the study area in wheat and rice cropping seasons from 2001 to 2017

| | Wheat cropping season | | | | Rice cropping season | | | |
|---|---|---|---|---|---|---|---|---|
| Year | $T_{min}$ (°C) | $T_{max}$ (°C) | $T_{mean}$ (°C) | Rainfall (mm) | $T_{min}$ (°C) | $T_{max}$ (°C) | $T_{mean}$ (°C) | Rainfall (mm) |
| 2001 | −5.60 | 30.70 | 10.56 | 414.90 | 9.50 | 36.50 | 24.26 | 855.90 |
| 2002 | −3.80 | 30.00 | 11.16 | 652.70 | 4.70 | 36.70 | 24.38 | 597.50 |
| 2003 | −5.90 | 30.50 | 10.07 | 447.40 | 9.70 | 39.50 | 24.72 | 512.40 |
| 2004 | −4.70 | 32.50 | 10.69 | 411.40 | 9.30 | 38.00 | 24.82 | 565.40 |
| 2005 | −6.10 | 33.30 | 10.56 | 377.70 | 7.90 | 36.80 | 25.38 | 530.20 |
| 2006 | −4.10 | 31.60 | 10.90 | 459.70 | 13.50 | 38.00 | 25.54 | 708.90 |
| 2007 | −2.20 | 32.20 | 12.13 | 361.70 | 10.30 | 38.70 | 25.26 | 723.20 |
| 2008 | −6.30 | 32.40 | 9.99 | 414.80 | 10.90 | 38.10 | 24.38 | 694.80 |
| 2009 | −8.10 | 34.80 | 10.09 | 390.40 | 10.00 | 37.10 | 24.48 | 933.20 |
| 2010 | −7.20 | 31.90 | 8.97 | 553.50 | 5.50 | 37.60 | 24.46 | 922.60 |
| 2011 | −7.20 | 35.50 | 9.19 | 169.50 | 9.60 | 37.60 | 23.58 | 825.90 |
| 2012 | −6.10 | 32.40 | 9.96 | 397.90 | 7.40 | 37.90 | 24.08 | 451.60 |
| 2013 | −5.90 | 31.90 | 9.46 | 492.70 | 7.10 | 39.20 | 25.16 | 713.80 |
| 2014 | −6.10 | 35.30 | 10.44 | 392.30 | 9.70 | 35.80 | 23.36 | 883.70 |
| 2015 | −5.20 | 31.60 | 10.24 | 430.70 | 9.00 | 38.50 | 23.70 | 1200.10 |
| 2016 | −9.40 | 29.80 | 10.62 | 594.60 | 9.60 | 39.00 | 24.96 | 1474.60 |
| 2017 | −4.30 | 33.70 | 11.19 | 371.70 | 6.00 | 39.30 | 25.02 | 869.40 |

The species diversity of the weed soil seed bank was measured with the following indexes:

Shannon–Wiener index $(H') = -\sum_{i=1}^{s}\left(\frac{n_i}{N}\right)\ln\left(\frac{n_i}{N}\right)$;[21]

Simpson index $(\lambda) = 1 - \sum_{i=1}^{s}\left(\frac{n_i}{N}\right)^2$;[22]

Ecological dominance $(C) = \sum_{i=1}^{s}\frac{n_i(n_i-1)}{N(N-1)}$,[20]

where $S$ is the total number of species in the community, $n_i$ is the number of individuals in species i, and $N$ is the total number of seeds in all species in a treatment.

Evenness index $(E) = \frac{N_2-1}{N_1-1}$,[20]

where $N_1 = e^{H'}$, $N_2 = \lambda^{-1}$; $H'$ is the Shannon diversity index and $\lambda$ is the Simpson diversity index.

One-way analysis of variance (ANOVA) and least significant difference (LSD) tests of the values of density and species diversity of the weed soil seed bank were conducted using SPSS 18.0 (IBM, Armonk, NY, USA), and figures were mapped using Origin 8.0 (OriginLab, Hampton, MA, USA). To visualize annual variations in species density and composition among the different treatments, nonmetric multidimensional scaling (NMDS)[23] was performed with the PC-ORD package. Before ordination, RD values were log($x$ + 1) transformed, and a distance matrix was calculated by the Bray–Curtis distance metric. Hence, 249 runs of the ordination with a maximum of 400 iterations per run were performed with an instability criterion of 0.00001. These runs were compared with 50 randomized runs to assess the significance of the reduction in stress from six dimensions to one. The IV values of weed species were used to determine the dominant species under different treatments, and the annual variations in the IV values of the dominant species were regressed and mapped using Origin 8.0. Multivariate analysis of variance (MANOVA) were conducted to determine the effects of weed control measure (as independent variables) and climatic factors, such as the minimum, maximum and mean temperature and rainfall (as covariates) of the study area in wheat and rice cropping seasons from 2001 to 2017, on the seed density of total and dominant species of wheat and rice associated weeds at a significance level of 5% by using SPSS 18.0 (IBM).

# 3 RESULTS

## 3.1 Annual variations in weed species and weed groups in soil seed banks

Under three treatments, a total of 42 species belonging to 19 families were recorded in the soil seed bank from 2001 to 2017 (Fig. 1). Of these species, there were ten species belonging to Poaceae, five species belonging to Cyperaceae, five species belonging to Scrophulariaceae, and four species belonging to Asteraceae; 21 species representing 11 families were wheat field weeds; and 21 species representing ten families were paddy field weeds. Fourteen species, *Echinochloa crus-galli*, *Leptochloa chinensis*, *Polypogon fugax*, *Schlerochloa kengiana*, *Cyperus difformis*, *Lindernia procumbens*, *Mazus japonicus*, *Veronica anagallis-aquatica*, *Veronica peregrina*, *Ammannia multiflora*, *Monochoria vaginalis*, *Myosoton aquaticum*, and *Chenopodium serotinum*, were recorded in all treatments from 2001 to 2017.

In the unweeded CK soil seed bank of the rice–wheat cropping field, broadleaf weeds (by density proportion) were initially dominant, but within 5 years, they were overtaken by grasses and eventually by sedges that increased yearly (Fig. 2). However, under the CH and HW treatments, the density proportion of the three weed groups remained relatively stable; broadleaf weeds were always dominant and followed by grass weeds. Of the annual variations in the density proportion of different weed groups, the density of broadleaf weeds was negatively correlated with grass weeds; that is, the density of broadleaf weeds decreased when the density of grass weeds increased, and *vice versa*. Additionally, the density of broadleaf weeds had a slight downward trend, while the density of sedge weeds slightly increased yearly.

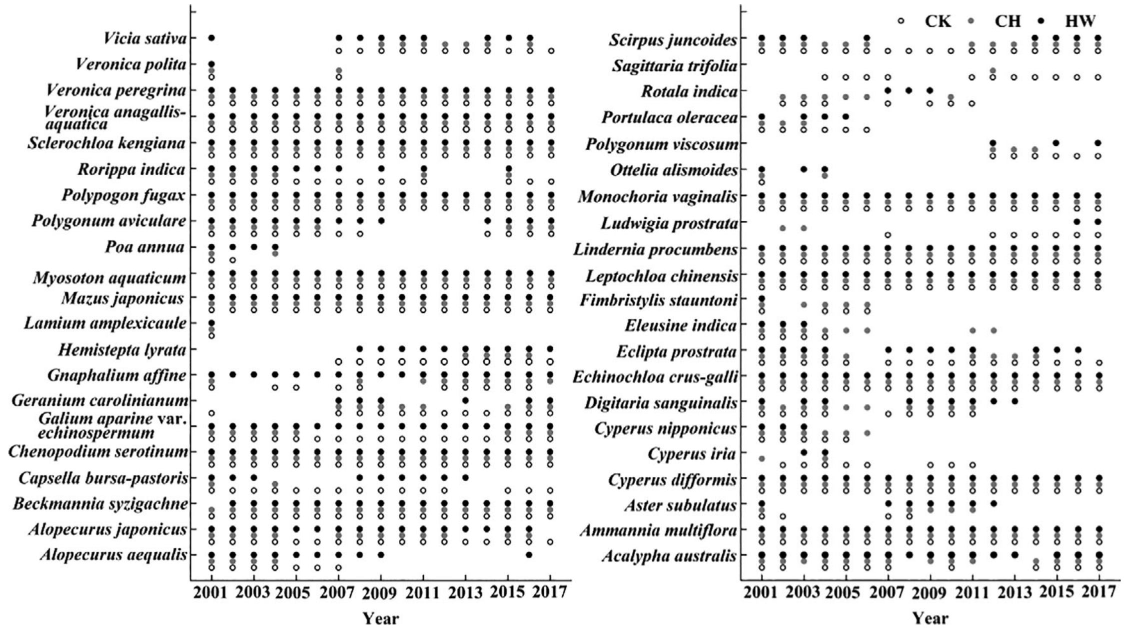


**Figure 1.** Weed species of the soil seed banks and occurrence year in different treatments.

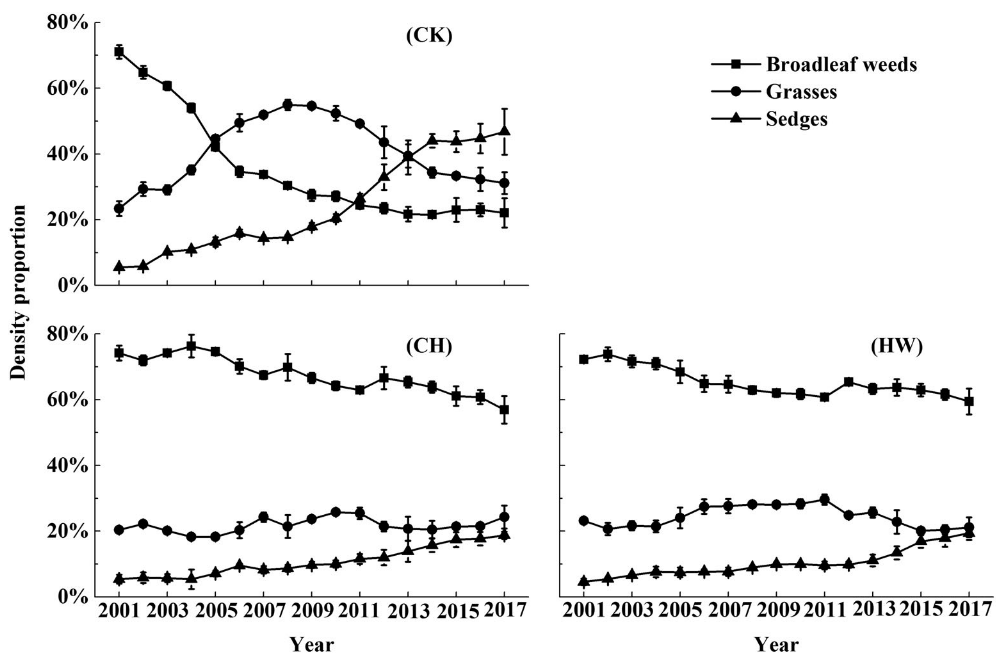


**Figure 2.** Annual variations in the density proportion of the weed groups in the different treatments: CH, HW and CK represent chemical herbicide, hand weeding and no weeding.

## 3.2 Weed community succession and the annual variation in dominant species

The IV values were used to determine the dominant weed species of the soil seed bank under different treatments in 2001 and 2017. Our results indicated that a composite exponential model, Exp3p2 taking the form $IV(y) = e^{a+b\cdot y+c\cdot y^2}$ (where IV indicates the IV value of weed species, $y$ is the last year of one treatment, and $a$, $b$ and $c$ are parameters controlling the shape and scale of the model), well fitted the annual variation in IV values of all dominant weed species under all treatments ($R^2 \geq 0.85$). In the unweeded rice–wheat cropping field (Fig. 3), the total IV value proportion of wheat weed species in the soil seed bank was 71.16% in 2001 but decreased to 33.66% in 2017. The dominant weed species of the wheat weed community in the soil seed bank were *Myosoton aquaticum*, *Mazus japonicus*, *P. fugax*, *V. peregrine* and *V. anagallis-aquatica* in 2001, which succeeded in *Galium aparine* var. *echinospermum*, *B. syzigachne*, *V. anagallis-aquatica*, *Mazus japonicus*, and *P. fugax* in 2017. Among these species, the IV values of most species decreased yearly, except for *G. aparine* var. *echinospermum* and *B. syzigachne*. The total IV value proportion of rice weed species in the soil seed bank was 28.84% in 2001 but increased to 66.34% in 2017. The wheat weed community in the soil seed bank



  

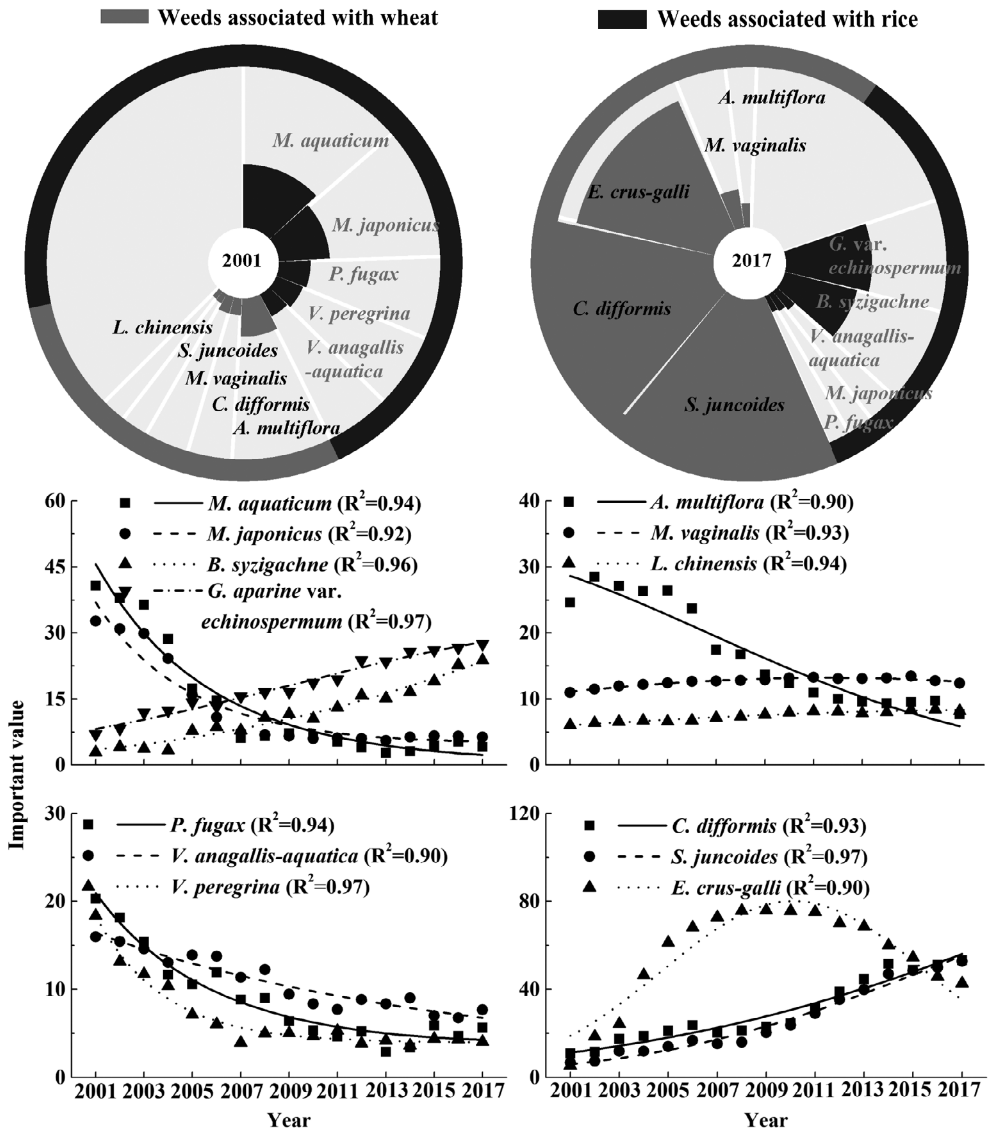


**Figure 3.** Weed community succession and annual variation in dominant weed species in unweeded rice–wheat cropping fields.

was dominated by *A. multiflora*, *Cyperus difformis*, *Monochoria vaginalis*, *Scirpus juncoides* and *Leptochloa chinensis* in 2001 succeeded in *Scirpus juncoides*, *Cyperus difformis*, *Echinochloa crus-galli*, *Monochoria vaginalis* and *A. multiflora* in 2017. Among these species, the *IV* values of *A. multiflora* decreased, that of *Cyperus difformis* and *Scirpus juncoides* increased yearly, the annual variation in the *IV* values of *Monochoria vaginalis* and *Leptochloa chinensis* showed a slightly increasing trend, and the *IV* values of *Echinochloa crus-galli* continuously increased for the first several years and then started to decrease.

In the CH rice–wheat cropping field (Fig. 4), the total IV value proportion of wheat weed species in the soil seed bank decreased from 74.26% in 2001 to 51.67% by 2017. The weed community associated with wheat in the soil seed bank, dominated by *Myosoton aquaticum*, *Mazus japonicus*, *V. peregrina*, *Chenopodium serotinum* and *Schlerochloa kengiana* in 2001, changed to a weed community dominated by *B. syzigachne*, *V. anagallis-aquatica*, *Mazus japonicus*, *Myosoton aquaticum* and *V. peregrina* in 2017. Of these dominant species, the IV values of *Myosoton aquaticum*, *Mazus japonicus*, *Chenopodium serotinum* and *Schlerochloa kengiana* continuously decreased in the first several years and then remained relatively stable. The IV values of *V. peregrina* were relatively stable in the first few years and then started to decrease, while those of *V. anagallis-aquatica* and *B. syzigachne* increased yearly. The total IV value proportion of rice weed species in the soil seed bank increased from 25.74% in 2001 to 48.33%. The dominant weed species of the wheat weed community in the soil seed bank were *A. multiflora*, *Cyperus difformis*, *Monochoria vaginalis*, *Leptochloa chinensis* and *Lindernia procumbens* in 2001 succeeding in *Cyperus difformis*, *A. multiflora*, *Monochoria vaginalis*, *Lindernia procumbens* and *Leptochloa chinensis* in 2017. Among these species, the IV values of *Cyperus difformis* and *Monochoria vaginalis* increased yearly, those of *A. multiflora*, *Lindernia procumbens* and *Echinochloa crus-galli* continuously increased for the first several years and then started to decrease, and those of *Leptochloa chinensis* continuously increased and remained relatively stable.

In the HW rice–wheat cropping field (Fig. 5), the total IV value proportion of wheat weed species in the soil seed bank was 73.52% in 2001, but it decreased to 52.70% in 2017. The weed

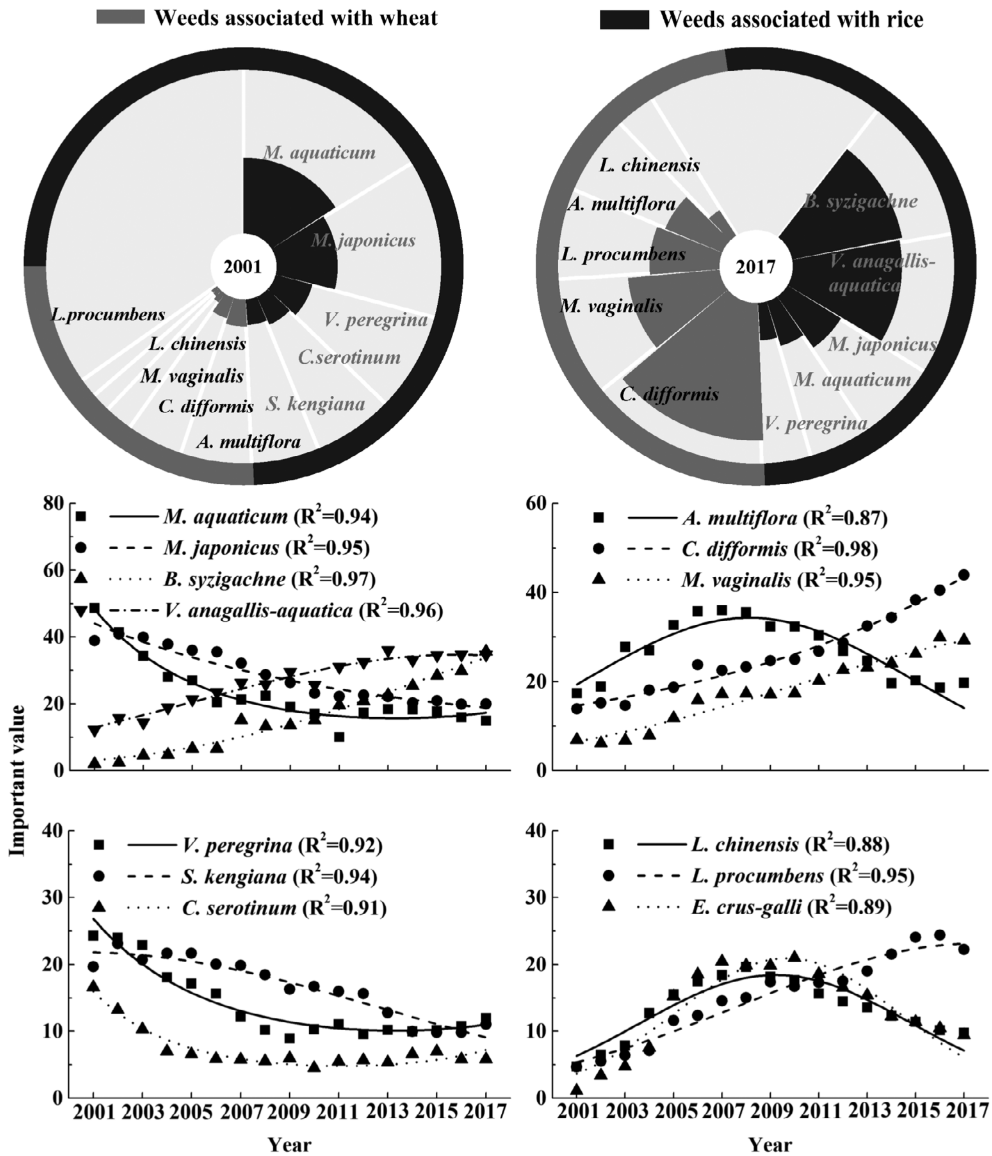


**Figure 4.** Weed community succession and annual variation in the dominant weed species in the chemical herbicide application rice–wheat cropping field.

community associated with wheat in the soil seed bank was dominated by *Myosoton. aquaticum*, *Mazus japonicus*, *V. peregrina*, *P. fugax*, and *Alopecurus japonicus* in 2001 and changed to *B. syzigachne*, *G. aparine* var. *echinospermum*, *Myosoton aquaticum*, *Mazus japonicus* and *V. anagallis-aquatica* in 2017. Among these species, the IV values of *Myosoton aquaticum* and *P. fugax* continuously decreased in the first several years and then remained relatively stable. The IV values of *Mazus japonicus* and *V. peregrina* decreased yearly, the IV values of *V. anagallis-aquatica* slightly increased for the first several years and then started to decrease, and the IV values of *B. syzigachne* and *G. aparine* var. *echinospermum* increased yearly. The total IV value proportion of rice weed species in the soil seed bank increased from 26.48% in 2001 to 47.30% in 2017. The weed community associated with rice in the soil seed bank, dominated by *A. multiflora*, *Cyperus difformis*, *Monochoria vaginalis*, *Leptochloa chinensis* and *Lindernia procumbens* in 2001, changed to a weed community dominated by *Cyperus difformis*, *Monochoria vaginalis*, *A. multiflora*, *Echinochloa crus-galli* and *Lindernia procumbens* in 2017. Of these dominant species, the IV values of *A. multiflora*, *Leptochloa chinensis*, *Echinochloa crus-galli* and *Lindernia procumbens* continuously increased for the first several years and then started to decrease, while the values for *Cyperus difformis* and *Monochoria vaginalis* increased yearly.

Based on above results, in all treatment plots, the weed community associated with wheat was dominated by *Myosoton aquaticum*, *Mazus japonicus*, *B. syzigachne*, *G. aparine* var. *echinospermum*, *P. fugax*, *V. anagallis-aquatica*, *V. peregrina*, *A. japonicus*, *Chenopodium serotinum* and *Schlerochloa kengiana* and the weed community associated with rice was dominated by *A. multiflora*, *Monochoria vaginalis*, *Leptochloa chinensis*, *Scirpus juncoides*, *Cyperus difformis*, *Echinochloa crus-gali*, and *Lindernia procumbens* in the soil seed bank from 2001 to 2017. Seed density of the dominant weed species in 2001 and 2017 and their mean annual variation from 2001 to 2017 in unweeded, CH application and HW rice–wheat cropping fields are presented in the Supporting Information, Table S1. Results of the MANOVA analyses (Tables 2 and 3) indicated that there was no significant effect of



  

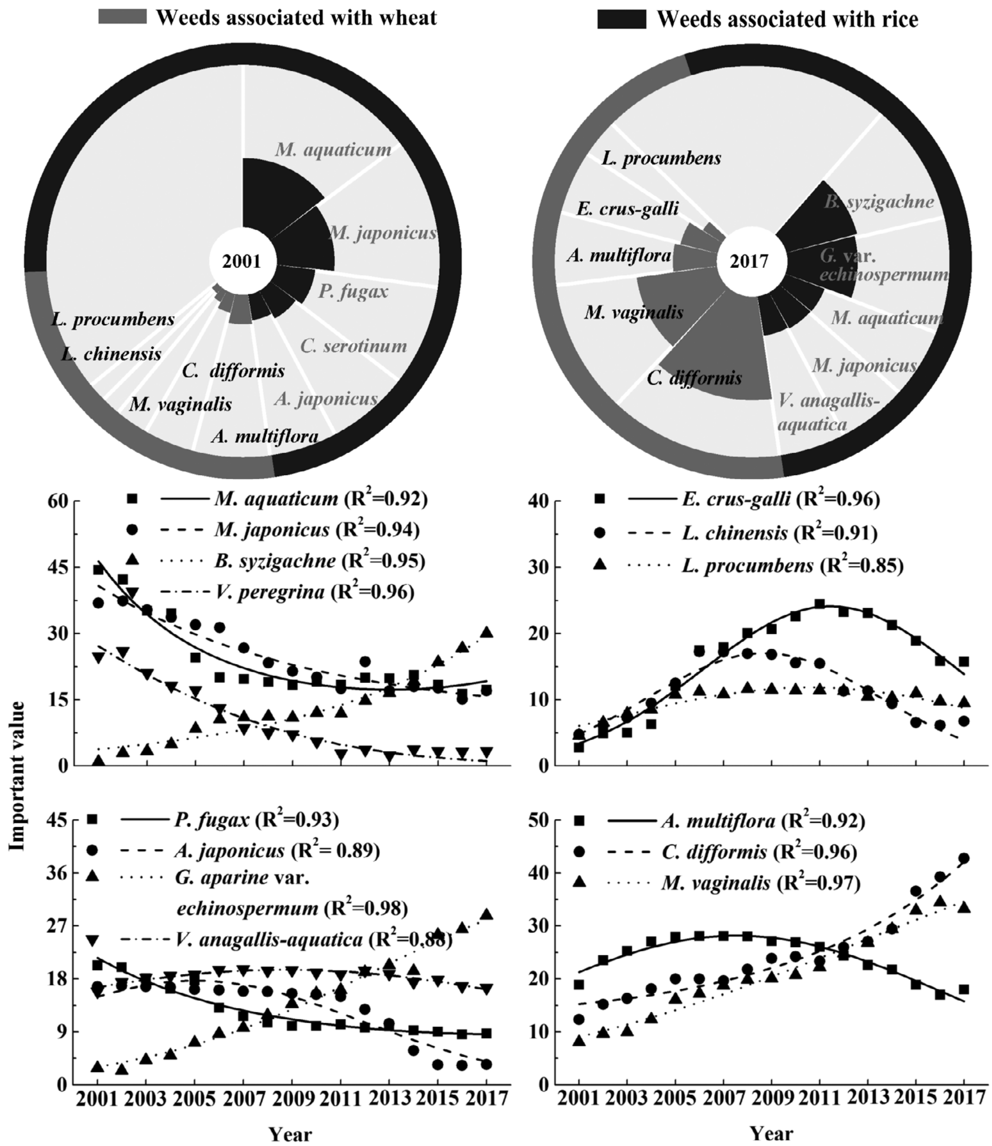


**Figure 5.** Weed community succession and annual variation in the dominant weed species in the hand weeded rice–wheat cropping field.

weed control measure on total seed density of wheat associated weeds ($P$ = 0.163), while the seed density of *B. syzigachne* ($P$ = 0.000), *G. aparine* var. *echinospermum* ($P$ = 0.000) and *A. japonicus* ($P$ = 0.005) were significantly influenced by different weed control measures. The seed density of *Myosoton aquaticum* ($P$ = 0.004), *Mazus japonicus* ($P$ = 0.003), *P. fugax* ($P$ = 0.004), *V. anagallis-aquatica* ($P$ = 0.010), *V. peregrina* ($P$ = 0.003), *Chenopodium serotinum* ($P$ = 0.004) and *Schlerochloa kengiana* ($P$ = 0.013) and total seed density of wheat associated weeds ($P$ = 0.004) were significantly influenced by the variation of the maximum temperature in wheat cropping season from 2001 to 2017. There were significant effects of weed control measure on seed density of *A. multiflora* ($P$ = 0.014), *Monochoria vaginalis* ($P$ = 0.000), *Leptochloa chinensis* ($P$ = 0.007), *Scirpus juncoides* ($P$ = 0.000), *Cyperus difformis* ($P$ = 0.000) and *Echinochloa crus-gali* ($P$ = 0.000) and total seed density of rice associated weeds ($P$ = 0.000). The seed density of *A. multiflora* ($P$ = 0.010), *Leptochloa chinensis* ($P$ = 0.012) and *Lindernia procumbens* ($P$ = 0.020) and total seed density of rice associated weeds ($P$ = 0.037) were significantly affected by the variation of rainfall in rice cropping season from 2001 to 2017.

### 3.3 Annual variation in species composition, total density and diversity

NMDS is an ordination method that maximizes rank order correlation between distances derived from the original data set and those in ordination space, and it is often the most appropriate ordination method for community data sets.[23] The NMDS ordination of RD values of the soil seed bank recommended a two-dimensional solution (minimum stress = 7.29, $P$ = 0.004), which showed a clear distinction in seed bank density and composition among different treatments from 2001 to 2017 (Fig. 6). The species density and composition of the seed bank in the CK field significantly differed from those in the other treatments after 1 year of applying herbicide and HW. The species density and composition of the seed bank were initially similar to those of the CH and HW fields but changed directions after 3 years. The total density of the soil seed bank showed a downward trend in all treatments

  

**Table 2.** Tests of between-subjects effects on the seed density of total and dominant species of wheat associated weeds

| | Effects on weed species (*P*-value) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | Total | SP1 | SP2 | SP3 | SP4 | SP5 | SP6 | SP7 | SP8 | SP9 | SP10 |
| Control measure | 0.163 | 0.744 | 0.964 | 0.000[A] | 0.000[A] | 0.418 | 0.238 | 0.872 | 0.005[A] | 0.829 | 0.247 |
| $T_{min}$ | 0.353 | 0.392 | 0.316 | 0.076 | 0.402 | 0.287 | 0.247 | 0.438 | 0.436 | 0.308 | 0.452 |
| $T_{max}$ | 0.004[A] | 0.004[A] | 0.003[A] | 0.152 | 0.152 | 0.004[A] | 0.010[a] | 0.003[A] | 0.072 | 0.004[A] | 0.013[a] |
| $T_{mean}$ | 0.916 | 0.944 | 0.957 | 0.513 | 0.397 | 0.934 | 0.912 | 0.978 | 0.506 | 0.906 | 0.988 |
| Rainfall | 0.318 | 0.331 | 0.322 | 0.787 | 0.782 | 0.370 | 0.371 | 0.301 | 0.529 | 0.295 | 0.534 |

Here, Total represents total seed density of wheat associated weeds and SP1, SP2, SP3, SP4, SP5, SP6, SP7, SP8, SP9 and SP10 represent seed density of *Myosoton aquaticum*, *Mazus japonicus*, *Beckmannia syzigachne*, *Galium aparine* var. *echinospermum*, *Polypogon fugax*, *Veronica anagallis-aquatica*, *V. peregrina*, *Alopecurus japonicus*, *Chenopodium serotinum* and *Schlerochloa kengiana*. The superscript letters a and A indicate significance at 5% and 1% level of probability.

**Table 3.** Tests of between-subjects effects on the seed density of total and dominant species of rice associated weeds

| | Effects on weed species (*P*-value) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source | Total | SP1 | SP2 | SP3 | SP4 | SP5 | SP6 | SP7 |
| Control measure | 0.000[A] | 0.014[a] | 0.000[A] | 0.007[A] | 0.000[A] | 0.000[A] | 0.000[A] | 0.610 |
| $T_{min}$ | 0.524 | 0.990 | 0.942 | 0.219 | 0.405 | 0.428 | 0.262 | 0.413 |
| $T_{max}$ | 0.343 | 0.078 | 0.126 | 0.225 | 0.110 | 0.724 | 0.826 | 0.659 |
| $T_{mean}$ | 0.664 | 0.238 | 0.665 | 0.273 | 0.138 | 0.396 | 0.712 | 0.999 |
| Rainfall | 0.037[a] | 0.010[a] | 0.110 | 0.012[a] | 0.079 | 0.929 | 0.384 | 0.020[a] |

Here, Total represents total seed density of rice associated weeds and SP1, SP2, SP3, SP4, SP5, SP6, and SP7 represent seed density of *Ammannia multiflora*, *Monochoria vaginalis*, *Leptochloa chinensis*, *Scirpus juncoides*, *Cyperus difformis*, *Echinochloa crus-gali*, and *Lindernia procumbens*. The superscript letters a and A indicate significance at 5% and 1% level of probability.

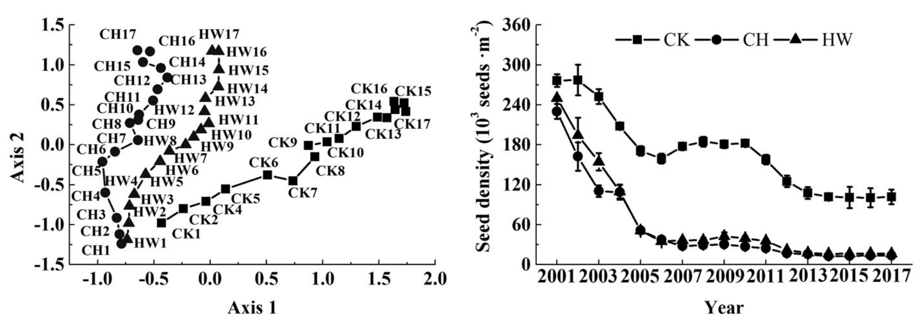


**Figure 6.** Annual variations in dominant species composition (left) and total density (right) in the different treatments; (left) CH, HW and CK represent chemical herbicide, hand weeding and no weeding and the numbers are the year of the experiment.

from 2001 to 2017, and the density decreased rapidly in the first several years and then tended to be relatively stable (Fig. 6). The total density of the soil seed bank in the CK rice–wheat cropping field was always higher than that in the other treatments during annual variation.

Long-term weeding in the rice–wheat system significantly affected the weed species diversity of the soil seed bank (Fig. 7). Among all treatments, the Shannon index and Simpson index of the soil seed bank were relatively stable in the first 3 years and then started to decrease rapidly in the CK rice–wheat cropping field, while in the CH and HW field, these indexes continuously increased in the first several years and then started to decrease, and they were always higher in the HW field than in the CH field during annual variations. The evenness index and ecological dominance index followed the opposite trend as the Shannon index and Simpson index, which were relatively stable in the first 3 years and then started to continuously decrease in the CK rice–wheat cropping field. However, in the CH and HW fields, they showed a downward trend in the first several years and then started to increase.

## 4 DISCUSSION

Farming practices and the interaction of weed populations in weed communities codetermine the dynamics of species composition in weed soil seed banks.[24] Our results indicated that in the rice–wheat cropping field with weeding pressure (CH and HW), the selection of weeding pressure on weed groups was stronger than the interspecific competition of weed species for environmental adaptation, which led to the density proportion of weed



  

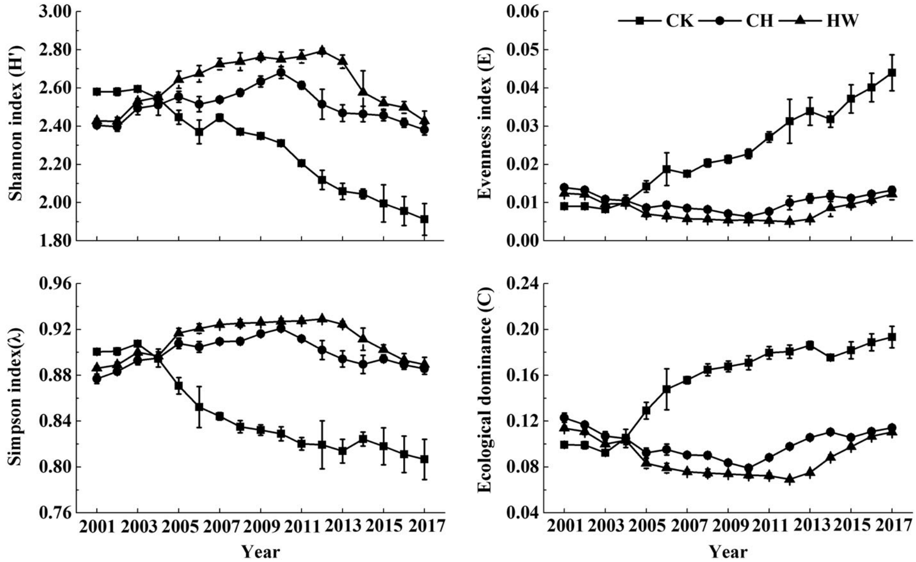


**Figure 7.** Annual variations in species diversity in different treatments.

groups in the soil seed bank remaining relatively stable, and the weed group was always dominated by broad leaf weeds. In the unweeded rice–wheat cropping field, the change in the dominant weed group was dominated by the interspecific competition of weed species for environmental adaptation. Because of the physiological similarity between grass weeds and crops (wheat and rice), grass weeds had a more competitive advantage than other weeds, and therefore, they rapidly increased to become the dominant weed group in the initial years. All the sedge weeds in our study were paddy weeds, so their seeds had higher adaptive ability than that of the grass weeds and broadleaf weeds in wheat field, enabling the weeds to survive the flooding environment of the rice-planting season; seeds of sedge weeds accumulated yearly, and eventually, sedge weeds overtook grass weeds and broad leaf weed to become the dominant weed group in the soil seed bank of the rice–wheat cropping field. Similarly, as the result of interspecific competition, the density of broadleaf weeds had a slight downward trend, while the density of sedge weeds slightly increased yearly in the weeding fields (CH and HW).

Previous studies have indicated that the seed density and species richness of a soil seed bank always declines with the succession of grassland,[25] derelict land[26] and forest,[27] and the variation in seed density and species richness of a soil seed bank was similar to that of surface vegetation during succession.[28] Our results demonstrated that the rice–wheat cropping system favoured the spread of paddy field weeds and that the seed proportion of paddy field weeds increased while that of wheat field decreased in both weeded and unweeded soil seed banks from 2001 to 2017. Water is a powerful selective agent for weed management in paddy fields,[29] and flooding is a key factor influencing the severity of weed competition.[30] The flooding environment of the rice–wheat cropping system rotted the seeds of *Capsella bursa-pastoris* or made them inactivate[31] but stimulated the emergence and growth of *Cyperus* spp., *Ammannia* spp. and *Monochoria vaginalis*.[32,33] Weeding had little effect on the composition of the dominant weed community in the paddy fields but significantly influenced the seed density of most dominant weeds and total rice associated weeds. Correspondingly, although the dominant weed species of the wheat fields differed greatly between unweeded and weeded fields, the total seed density of wheat associated weeds was not significantly affected by weeding. The annual IV value variation in *Cyperus difformis* and *Leptochloa chinensis* in 2001 and *Echinochloa crus-galli* showed a similar pattern of change in both weeded and unweeded fields from 2001 to 2017. Relatively short weed species such as *V. anagallis-aquatica*, *Cyperus difformis*, *Monochoria vaginalis* and *Lindernia procumbens* in CH field and *G. aparine* var. *echinospermum*, *Cyperus difformis* and *Monochoria vaginalis* in the HW field showed an upward trend in annual IV variation that might be because these species needed less weeding than other weed species.

Tillage has a major influence on the vertical distribution of weed seeds in arable soils,[34] and this pattern of seed distribution has a critical effect on seed germination and survival.[35] Fertilizer application significantly changes the density of a soil weed seed bank, the diversity index, and the community structure.[36] In this study, under the effect of tillage and fertilization, the total density of the weed soil seed bank significantly decreased in the unweeded field; there was a similar pattern of change in total density of the weed soil seed bank but different species density and composition of soil seed bank between CH and HW fields. Our results indicated that the seed density of wheat and rice associated weeds could also be influenced by annual variation of the maximum temperature and rainfall in wheat and rice cropping seasons. The maximum temperature of wheat cropping season is in May at the maturity period, therefore the maximum temperature might be possibly related to the seed maturity and production of the weed species such as *Myosoton aquaticum*, *Mazus japonicus*, *P. fugax*, *V. anagallis-aquatica*, *V. peregrina*, *Chenopodium serotinum* and *Schlerochloa kengiana*. Water, as flooding, is always used to manage weeds in rice field. Previous studies demonstrated that the seedling emergence of *Ammannia* spp. and *Leptochloa chinensis* decreased with increased water depth[37,38] and accordingly seed density of these weed species in the soil would be influenced by rainfall variation. Numerous studies have demonstrated that the biodiversity of weed species generally declines in response to herbicide application.[39–41] However, these are studies that cover short periods or relate only to the weed species of one crop season and not the weed species of the whole crop rotation system. In this study, the application of herbicide and HW slightly increased weed species diversity and decreased weed community evenness and dominance in the first several years but could have negative consequences over the long term. In the unweeded field, without the selection pressure of weeding, weed species diversity increased; weed community evenness and dominance decreased in the first 3 years, but they showed opposite trends of change after that time.

Overall, our study demonstrated that the long-term application of CH had a similar effect as HW, which could maintain the weed community in a relatively benign and stable structure for a long time. Some species involved in this study have been reported to be malignant herbicide-resistant weed species that are hard to control, such as the wheat weed species *B. syzigachne*, *A. japonicus*, *Schlerochloa kengiana* and *G. aparine* var. *echinospermum*[42–44] and the paddy weed species *Echinochloa crus-galli*, *Leptochloa chinensis* and *Monochoria vaginalis*,[45–47] due to the long-term application of a single herbicide. The transformation of these weed species to malignant and herbicide-resistant weeds was mostly due to the long-term application of post-emergence herbicide, which is different from pre-emergence herbicide in our study. Furthermore, those concerned with herbicide resistance should also consider the mobility of herbicide resistance via weed seed dispersal or pollen flow, which is the influence of herbicide-resistant weed species in neighbouring fields or farms, when developing weeding strategies and plans.[48] However, these herbicides were dispersed into the experimental plots, and resistant weeds to post-emergence herbicides were still controlled by pre-emergence herbicides.

Currently, a total of 161 grasses have evolved resistance to acetolactate synthase (ALS) inhibitor herbicides (http://www.weedsience.org), and the resistance of broadleaf weeds[49,50] and sedges[51] to bensulfuron methyl has been widely reported in rice growing areas of the world. Seventeen cases of isoproturon-resistance weeds in different crops have been reported from 1982 to 2009, and butachlor- and acetochlor- resistance cases have also been found in rice and corn and soybean fields (http://www.weedsience.org). The incidences of resistance of bensulfuron methyl,[52] isoproturon[53] and butachlor[54] are the result of over 10 to 12 years of their continuous use as post-emergence herbicides. However, in our study, the resistant weed population had not yet evolved when the above herbicides were reasonably applied as pre-emergence herbicides for 17 years. Similarly, a simulation model predicted that herbicide resistance occurred at the fifth years when post-emergence herbicides were used as the sole herbicide treatment, while no herbicide resistance appeared when a short-acting pre-emergence herbicide was continuously applied for 15 years, which could be explained by the lower seasonal kill rate of pre-emergence herbicides.[55]

## 5 CONCLUSION

Our results indicated that the rice–wheat cropping system favoured the spread of paddy field weeds and that the seed proportion of paddy field weeds increased while that of wheat field decreased in both weeded and unweeded soil seed banks. Weeding had little effect on the composition of the dominant weed community in the paddy fields but had a great influence on that in the wheat fields. In the unweeded rice–wheat cropping field, species diversity decreased and weed community evenness and dominance increased. Broadleaf weeds were initially dominant but were overtaken by grass weeds and eventually by sedge weeds. The annual variations in the density proportion of the weeded groups and species diversity in the rice–wheat cropping field with long-term application of pre-emergence chemical herbicides were similar to those in the hand weeding treatment, which maintained the weed community in a relatively benign and stable structure with a lower degree of harm. Consequently, reasonable application of pre-emergence herbicide, especially the alternative application of different herbicides should be adopted when developing long-term weeding strategies and plans to maintain the weed community at a durable relatively low infestation level.

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## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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