

## Article

# Rice Paddy Soil Seedbanks Composition in a Mediterranean Wetland and the Influence of Winter Flooding

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**Abstract:** Soil seedbanks are defined in composition and quantity by many environmental factors inherent to a specific area, and they can be an indicator of the potential problems of weeds in crops. In Valencia (Spain), rice is cultivated with continuous flooding during the growing season, and after harvesting, many of the paddy fields are flooded again during the winter. This study investigates the paddy fields' soil seedbank composition in this Mediterranean paddy area and the effect of winter flooding on the soil seedbank. Multispectral images from the Sentinel-2 satellite were used to characterise the water level of paddies in winter. Satellite images facilitated the characterisation of winter flooding in fields. Soil samples from sixty-nine points distributed over 15,000 ha of paddies were used to determine the composition of the seedbank plots. The data were spatially represented by geographic information systems. The species that contributed most to the paddy seedbank were *Cyperus difformis* L., an important rice weed in the Mediterranean area, and other rice weeds such as *Echinochloa* sp. and *Leptochloa fusca* subspecies. Other species with a great contribution to the seedbank are species that develop in paddy fields that produce a large quantity of small seeds, such as *Lemna* sp., *Polypogon monspeliensis* (L.) Desf., and *Nasturtium officinale* R. Br. These species interfere little or do not interfere with the rice crop. The study revealed that in general, flooding reduced seedbank density with differences between species. Furthermore, the influence of winter flooding on the different plant species obtained as well as their distribution maps are a further step in this protected area from the point of view of weed management in rice crop, as well as in the management of this Mediterranean wetland.



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**Keywords:** rice weeds; Sentinel-2; multispectral images; remote sensing; seed germination; *Cyperus difformis*; *Echinochloa*; *Leptochloa fusca*; Albufera

## 1. Introduction

Most of the weeds that can appear in cultivated fields are found in the soil in the form of seeds or propagules, which constitutes the soil seedbank. Knowledge of this seedbank is consequently an aspect of great interest when predicting weed seedling populations and planning strategies of weed control and management programs [1–4]. Several factors influence the seedbank diversity and density, such as soil properties, climate features, predators, and crop management [5–7].

The seeds of some species remain viable in the soil for short periods, but there are some seed species that can remain latent (dormant) in soils and germinate after long periods. Factors that can influence dormancy can be genetic, environmental, or biological [8], and changes in environmental conditions affect the dormancy of seeds and their viability. A clear example is the water regime in the field. Fluctuations in soil moisture, water level in fields, irrigation strategies, and flooding duration are some of the parameters that affect the dormancy and viability of seeds, and therefore, the germination efficiency and the final seedbank composition and density. This can be observed in *Leptochloa fusca* [L.] Kunt ssp. *fascicularis* [Lam] N. Snow (bearded sprangletop, LEFFA), *Echinochloa crus-galli*, or

*Cyperus difformis*, among others, in which water management has a positive effect on the subsequent seed germination and development [8–12].

Rice (*Oryza sativa* L.) is a crop that requires a significant amount of water for its cultivation. In the Valencia rice-growing area, located east of the Iberian Peninsula (Spain), more than 15,000 ha are cultivated in a coastal wetland called Albufera de Valencia, a protected RAMSAR area due to its great environmental interest and for being a wintering area for waterfowl in Spain. Rice is cultivated in monoculture, not rotated with any other crop. The cultivation period extends from May to early October and flood conditions are quite uniform in all plots. Paddy fields remain flooded from early May to late August or early September before harvesting.

After harvesting, fields are refilled with water and remain flooded until mid-winter (winter flooding). This is a traditional practice that started for waterfowl hunting purposes. At present it is being used to expand favourable habitats for migratory birds and due to the benefits that winter flooding produces in the rice fields [13,14]. In the lower areas close to the Albufera lagoon and the sea, water levels can exceed 1 m in depth during the winter flooding. The water level decreases with the distance to the lake and the sea and due to differences in terrain elevation and water scarcity, areas with higher levels, farther from the Albufera lagoon and the coast, are not completely flooded and remain dry during the autumn–winter period. These modifications between paddy fields in the flood conditions that occur in autumn–winter can influence the species that develop during those months and the seedbank. Additionally, flooded rice fields attract waterfowl that feed on plants and seeds from flooded and moist soil, influencing the seed bank composition [13–16].

In rice cultivation, the development of different weeds is influenced by the water level in plots [12,17]. Important weed species that are frequently found in flooded production systems include *Echinochloa* complex (barnyard grass, 1ECHG), LEFFA, and *Cyperus difformis* L. (small flower umbrella sedge, CYPDI) [18]. In general, higher water levels decrease the diversity and growth of weeds [11,18,19]. Water may also be responsible for the dissemination and introduction of weed propagules in fields [18,20,21]. In conditions of saturation but not flooding, species that do not develop well with flooding, such as *Leptochloa fusca* (L.) Kunth ssp. *uninervia* (J. Presl) N. Snow (Mexican sprangletop, LEFUN) can grow. Contrarily, in non-flooded rice cultivation systems, it is observed that weeds common to other crops appear, such as *Digitaria* spp. (crabgrass, 1DIGG), *Portulaca oleracea* L. (common purslane, POROL), or *Cyperus esculentus* L. (yellow nutsedge, CYPES) [18,22]. In that regard, [12] reveal, in paddy fields in China, that the irrigation strategy (traditional flooding conditions versus intermittent) leads to the presence of some specific tolerant submerged or non-submerged weeds in rice fields.

The extension of the flooded area in winter flooding varies slightly from one year to the next, depending on the weather and the availability of water and the different conditions of the plots. Therefore, to determine the seedbank of each plot and changes in this, it is necessary to know the flooding conditions for each specific plot during the winter flooding period.

Satellite images are a valuable tool to indirectly study soil properties and vegetation, as well as their spatial and temporal variability. The combination of spectral bands to generate specific maps and indices or the use of more developed mathematical models tested with field data facilitates the identification of edaphic variables that are often difficult to observe and determine from the ground [23–25]. Some of the soil properties studied by means of multispectral images are water content, pH, organic matter content, salinity, and soil temperature. In the case of vegetation, multispectral images facilitate the definition of spatial distribution, state of development, productive potential, and plant health using specific indices [26,27]. In the case of weedy fields, multispectral images facilitate the identification and discrimination of areas occupied by weeds and the preparation of distribution maps, which is of great interest in studies of population dynamics and interaction with crops [28,29]. Soil properties that are consistent in the short term can be correlated with other properties that allow the design of specific strategies for efficient

weed management [30]. Many weed species are located consistently in the same areas within crop fields, especially in cereal-sown fields, where it has been seen that for some weeds, the distribution of weed seedlings mapped in one year is a good predictor of future seedling distribution [29,31].

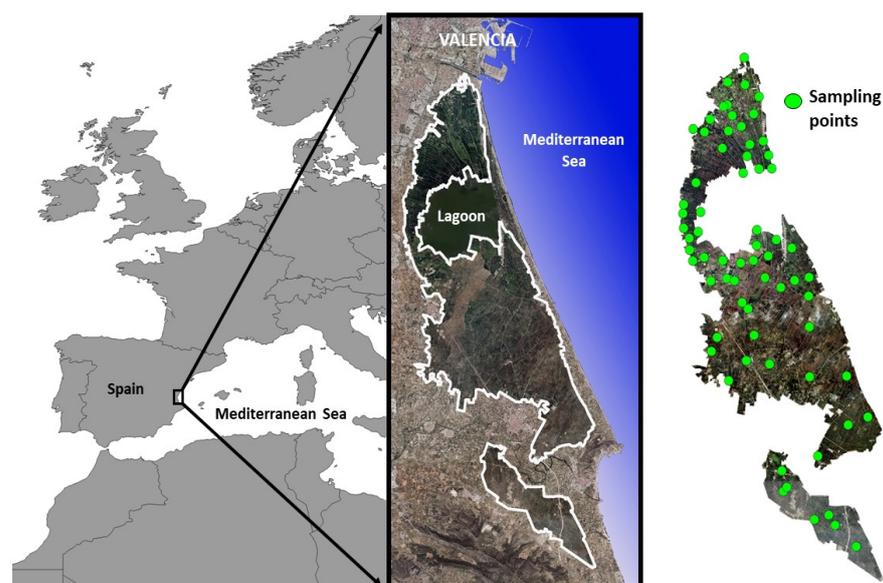
Because higher water levels in fields decrease diversity and growth of weeds, water level in fields during the autumn–winter period could affect the composition and density of the seedbank. Ref. [17] concluded that it was necessary to conduct detailed studies of the effects of winter floods on the life cycle of weeds. Given that the distribution of winter flooding in the fields of the Valencian rice zone is quite constant, it would be useful to describe the relationship between winter flooding and the seedbank diversity and density. This is useful in paddy fields from an agronomic and weed management point of view. Knowing the composition of the seedbank can help to assess potential weed problems by zones within this Mediterranean rice paddy. It is useful for the weed management of this area of agricultural and environmental value. Therefore, the objectives of the present research were (1) to characterise the seedbank of the paddy fields of this area before sowing rice; (2) to determine the extent and level of winter flooding using multispectral satellite images; (3) to define the effect of winter flooding on the seedbank and the main rice weeds; and (4) to prepare spatial distribution maps of the seedbank of the main weed species in paddy fields of the study area.

The novelty of this work is in the application of powerful technologies, such as multispectral satellite images and geographic information systems (GIS), that make it possible to build distribution maps of weed paddy seedbanks to relate the influence of soil flooding to the behaviour of weeds in an economically accessible and dynamic way. This dynamic application can be temporarily renewed at the beginning of each cultivation period and can establish guidelines for the management of an area of great agricultural and environmental value, such as the rice fields of Valencia.

## 2. Materials and Methods

### 2.1. Study Area

The rice area under study is a coastal wetland located south of the city of Valencia (eastern Spain) next to the Mediterranean Sea. The paddy area covers 15,000 ha between 39.42° N and 39.10° N latitude and −0.40° W and 0.24° E longitude (Figure 1).



**Figure 1.** Location of the study area (Albufera de Valencia) and sampling points.

Rice cultivation was carried out in a marsh area with saline soils containing carbonates of alluvial origin surrounding a central lagoon (Albufera) [32]. The rice cycle occurs

between May (flooding fields and sowing) and mid-October when the harvest ends. After harvesting, the paddy area is flooded again during the winter flooding period (October–mid winter). The climate is warm and it is classified as Mediterranean with an average temperature of 17.6 °C, 885 mm of evapotranspiration, and an annual rainfall of 540 mm, distributed during spring and autumn. Its semi-arid character, with mild winters and hot summers, together with low rainfall and high evapotranspiration, are the most limiting characteristics that define the hydrological management in the wetland as well as the management of paddy fields.

## 2.2. Sampling Process

Soil samples were taken from rice plots randomly distributed across the rice area from the end of March to the beginning of May 2019 to characterise the seedbank (Figure 1). Spring sampling seems more reliable in seedbank studies than at other times [2], which was consistent with the current study of the effect of winter flooding on the seedbank of rice, a spring sowing crop. During this period, the fields were dry and topsoil had been worked with a mouldboard plough in the first 20–30 cm. In each sample plot (field), the coordinates for georeferencing were taken with a tablet using Mide Maps Pro V.11.15.1 software (Blue Blink One, Castellón, Spain).

Soil sampling was carried out using a PVC cylinder 12.5 cm in diameter and 10 cm deep. More frequent paddy fields in the area are two to five ha in size. A diagonal transect that crossed the entire field was established according to the inflow and outflow of water. From each field, five subsamples were taken equidistant from the transect and subsequently mixed and labelled as a single sample. Sixty-nine plots distributed throughout the rice zone were sampled (Figure 1). In the laboratory, samples were left to air dry in plastic trays, and once dried the samples were crumbled with the help of a wooden roller. Next, they were labelled and stored in plastic bags for further processing.

## 2.3. Seedbank Determination

The characterisation of the seedbank was carried out by indirect methods, determining the seeds that germinate from the soil samples. The seedbank characterisation varies with the estimation method used. In addition, for each plant species the most appropriate method to characterize its presence in the seedbank also varies [33]. To characterise the seedbank of the paddy fields of Valencia as well as the most appropriate method to characterise the presence and distribution of the most important weeds, in this work two seedbank estimation methods were used: the tray method (TM) and extraction method (EM).

### 2.3.1. Tray Method (TM)

In a 13 × 13 × 5 cm<sup>3</sup> plastic tray with drainage holes, a 2 cm layer of seedbed substrate (Suliflor Propagation Substrate SF1 Radvilīškis, Lithuania) was placed and 150 g of the dry and disaggregated soil sample was spread on it. For each sample, four repetitions (four trays) were prepared, producing 276 trays. To apply irrigation or floodwater under the same dosage conditions, these individual trays were placed in other black trays longer than 60 × 30 × 10 cm<sup>3</sup>, which served as the basis for the application of the irrigation water. The trays with samples were placed in a climatic chamber (12/12 h light/dark cycle at 28/20 °C) for a period of 3 days to favour the start of germination. After 3 d, the trays were placed on benches in a glass greenhouse (30/20 °C day/night). Trays were kept in a saturated condition to maintain conditions close to those found in the rice fields. Four weeks after planting, the weed seedlings that emerged were identified, counted, and removed. After four weeks, seedlings were sprayed with a 1% solution of gibberellic acid to stimulate the germination of non-germinated seeds. Over the next two weeks, the plants that emerged were again counted and identified, and added to those previously counted to obtain the total number of plants emerging by species in each sample (i) and repetition (j) (abundance,  $A_{sij}$ ). For each sample and repetition, the total seedbank ( $A_{ij}$ ) was calculated as:

$$A_{ij} = \sum A_{sij} \quad (1)$$

For each of the sixty-nine plots, the abundance ( $A_i$ ) of the seedbank was calculated as the mean of the four  $A_{ij}$  of each plot.

### 2.3.2. Extraction Method (EM)

The second method for determining the seedbank was based on an adaptation of the method described by Barralis and Chadoeuf [5]. As in method 1, for each dry and disaggregated soil sample, 150 g was taken and placed in a beaker. A solution containing 50 g of sodium hexametaphosphate and 25 g of sodium bicarbonate per litre was added in the proportion of 2 mL g<sup>-1</sup> of soil, in order to break up the aggregates of the sample and release the seeds. The resulting mixture was stirred until a homogeneous suspension was obtained. Seeds were separated from the suspension by screening through 4 and 0.2 mm sieves and collecting the fraction that remained on the 0.2 mm sieve (containing a mixture of seeds and sand particles). This mixture was placed on a 13 × 13 × 5 cm<sup>3</sup> plastic tray with drainage holes in the base and a 2 cm layer of seedbed substrate as described in the TM method and the seedbank was characterised in the same way as described in the TM method. Four repetitions per sample were also prepared.

### 2.4. Calculation of the Frequency, Abundance, and Specific Contribution of Each Species

Once the  $A_{sij}$  and  $A_{ij}$  values were determined, the absolute frequency (FA), relative frequency (F), average abundance of each species (AM), and the species-specific contribution to the seedbank (Cs) were calculated according to the following equations:

$$F_s = 100 \times FAs/N \quad (2)$$

where N is the total number of fields and FAs = number of fields in which the species s was present.

$$AM_s = \Sigma A_{sij}/4 FAs \quad (3)$$

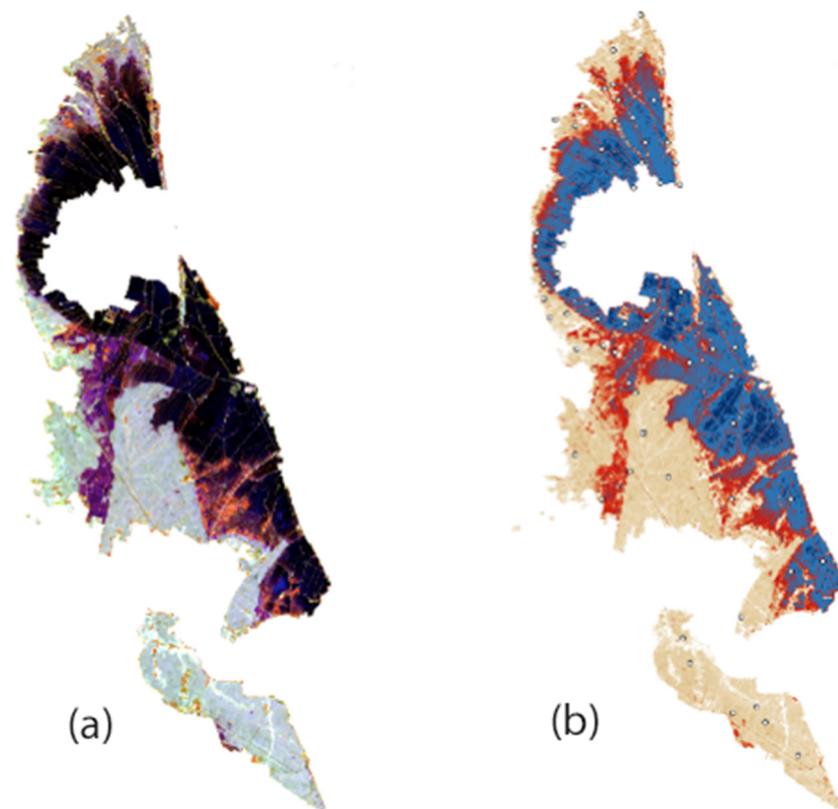
$$Cs = 100 \times AM_s/\Sigma (AM_s \times F_s) \quad (4)$$

### 2.5. Winter Flooding and Geostatistical Methods

Multispectral images from the Sentinel-2 satellite, obtained from the U.S. Geological Survey (<https://earthexplorer.usgs.gov/> (accessed on 12 April 2020)) were used to characterise the winter flood level of the rice fields (Figure 2a). Sentinel-2 multispectral images are comprised of 13 bands, called B01, B02..., B12, and an additional band B8A, which corresponds to the reflectance on the Earth's surface at different radiation intervals of different wavelengths. Images from various dates (Table 1) were used to delimit the area corresponding to the rice fields and the water levels of winter flooding.

**Table 1.** Sentinel-2 images used for paddy fields and winter flooding delimitation.

No.	Date	Function
1	15 July 2018	Describe the perimeter of paddy fields
2	1 January 2019	Calculate and classify winter flooding areas
3	27 March 2019	Soil samples



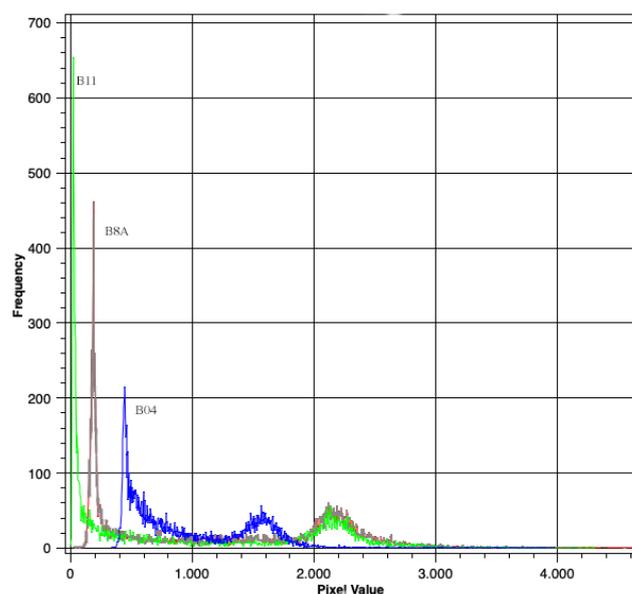
**Figure 2.** Images of the winter flooding level of paddy fields of the Valencian rice area: (a) combination of layers B8A, B11, and B04 from Sentinel-2 multispectral images (January 2019); (b) representation in false colour, with sampling points for seedbank estimation.

The QGIS geographic information system v.2.18.15 application was used to build a geographic information system (GIS) for the study area, locate the sampled fields, and characterise the level of winter flooding (WF). The following steps were taken:

- Phase 1 Delimitation of paddy field areas: With multispectral image No. 1, the rice-growing area was delimited by combining bands B8A, B11, and B02 (Figure 1). At this time, rice plants in Albufera de Valencia were between the final tillering phase and the beginning of stem elongation (BBCH 29-32);
- Phase 2 Characterisation of the winter flood area: The multispectral image No. 2 of January 2019 was used, corresponding to the moment when the paddy fields reached the highest levels of winter flooding. For this, layers B8A, B11, and B04 were combined and a false colour image was obtained, which was cut with a layer of polygons corresponding to the paddy fields obtained from Phase 1 (Figure 2a). Four levels of WF were defined (Table 2) from the graphic of the pixel values of the cropped image bands (Figure 3). These levels were represented with new colours to improve visualisation (Figure 2b);
- Phase 3 Assignment of flood level in sample plots: For each of the sixty-nine sampling points, the winter flood level was obtained by intersecting the sampling point layers with the flood level layer obtained in Phase 2 with GIS;
- Phase 4 Validation: The four winter flood levels were validated according to the assignment obtained in Phase 3 with real field data from the georeferenced plots in the area and whose winter flood level was previously known.

**Table 2.** Winter flooding levels of paddy fields constructed from B8A–B11–B04 layer combinations of Sentinel-2 images from 1 January 2019.

Pixel Value	Flooding Level	Description
0–150	1	Flooding
150–170	2	Flooding
170–1200	3	Puddles or Saturated
1200–1900	4	Dry
>1900	-	No crop

**Figure 3.** Pixel values of bands B8A, B11, and B04 of paddy fields in Valencia obtained from 1 January 2019 from Sentinel-2 multispectral images.

## 2.6. Models

A two-part model was used to analyse the results. Analyses were carried out with Statgraphics Centurion XVII v.2.04 (Statgraphics Technologies, Inc., The Plains, VA, USA). First, the most suitable method for the estimation of the seedbank and the seedbank of each species was selected. To correct the right-skewness of the bank, a logarithmic transformation of the data was performed ( $\ln(A_{ij} + 1)$ ). A paired t-test was used to compare the total abundance estimate between methods (TM or EM) at sample level. For each of the found species a general linear model (GLM) was used to determine which of the two methods was more effective (higher number of estimated individuals). Second, the influence of winter flood grade on the seedbank was analysed using a concurrent regression model. For a better approximation, the data of the dependent variables were taken as ( $\ln(A_{ij} + 1)$ ), transformed from the data obtained by the EM used for total seedbank estimation.  $A_{ij}$  was replaced by  $As_{ij}$  for the estimation of influence of the winter flood grade on the seedbank of each species. To determine the distribution maps of plant species, the Empirical Bayesian kriging (EBK) method was used. EBK is a geostatistical interpolation method that has been used in several studies on vegetation and soils because it adjusts and reduces the standard error more precisely than other kriging methods. In addition, it reduces the effect that irregular sampling can produce over outcomes [34–38].

At species level, in those in which a significantly higher number of individuals was obtained in the germinable seedbank by TM, the number of individuals obtained was used to analyse the influence of winter flooding and the elaboration of distribution maps.

### 3. Results

Eighteen taxa were identified in the study area, of which seven were common rice-weed species in the Mediterranean paddy fields (Table 3). These species were CYPDI and *Polypogon monspeliensis* (L.) Desf. (POLMO), *Lemna* sp. (1LEMG), 1ECHG, *Heteranthera reniformis* Ruiz & Pav. (HETRE), *Leptochloa* sp. (LEFSS), and *Bergia capensis* L. (BERCA). In addition, *Oryza sativa* L. (ORYSA) was also present and included both cultivated rice and spontaneous and wild forms (weedy rice). A small number of plants of frequent species in the area were also found that do not develop in the rice paddies during the growing season and when fields are flooded. *Amaranthus* sp. (1AMAG), *Solanum nigrum* L. (SOLNI), *Senecio vulgaris* L. (SENVU), *Malva parviflora* L. (MALPA), *Portulaca oleracea* L. (POROL), and *Erodium malacoides* (L.) L'Hér (EROMC) are some of the plant species that usually appear in times when the fields are dry and without cultivation.

**Table 3.** Families, species, and number of viable seeds obtained by two estimation methods, try method (TM) and extraction method (EM), in the Valencia rice seedbank.

Family	Species	Germinable Seedbank (N°) <sup>1</sup>	
		TM	EM
Cyperaceae	<i>Cyperus difformis</i> L.	11,057 <sup>a</sup>	34,760 <sup>b</sup>
Poaceae	<i>Polypogon monspeliensis</i> (L.) Desf.	1281 <sup>a</sup>	1264 <sup>a</sup>
Araceae	<i>Lemna</i> sp.	227 <sup>a</sup>	520 <sup>b</sup>
Brassicaceae	<i>Nasturtium officinale</i> R.Br.	237 <sup>a</sup>	454 <sup>b</sup>
Poaceae	<i>Echinochloa</i> sp.	461 <sup>b</sup>	204 <sup>a</sup>
Poaceae	<i>Leptochloa</i> sp.	247 <sup>a</sup>	225 <sup>a</sup>
Ranunculaceae	<i>Ranunculus sceleratus</i> L.	175 <sup>a</sup>	160 <sup>a</sup>
Ranunculaceae	<i>Ranunculus peltatus</i> Schrank	66 <sup>a</sup>	106 <sup>b</sup>
Compositae	<i>Sonchus oleraceus</i> L.	37 <sup>a</sup>	47 <sup>a</sup>
Pontederiaceae	<i>Heteranthera reniformis</i> Ruiz & Pav.	66 <sup>a</sup>	2 <sup>b</sup>
Elatinaceae	<i>Bergia capensis</i> L.	55 <sup>a</sup>	3 <sup>b</sup>
Amaranthaceae	<i>Amaranthus</i> sp.	6 <sup>nd</sup>	4 <sup>nd</sup>
Poaceae	<i>Oryza sativa</i> L.	19 <sup>nd</sup>	0 <sup>nd</sup>
Solanaceae	<i>Solanum nigrum</i> L.	1 <sup>nd</sup>	2 <sup>nd</sup>
Compositae	<i>Senecio vulgaris</i> L.	3 <sup>nd</sup>	1 <sup>nd</sup>
Malvaceae	<i>Malva parviflora</i> L.	0 <sup>nd</sup>	3 <sup>nd</sup>
Portulacaceae	<i>Portulaca oleracea</i> L.	0 <sup>nd</sup>	2 <sup>nd</sup>
Geraniaceae	<i>Erodium malacoides</i> (L.) L'Hér	1 <sup>nd</sup>	0 <sup>nd</sup>
	Other/non identified	607 <sup>nd</sup>	771 <sup>nd</sup>
	Total	14546 <sup>a</sup>	38528 <sup>b</sup>

<sup>1</sup> different letters indicate significant differences ( $p < 0.05$ ); ns = not significant; nd = not determined.

The same number of species (16) was obtained using the two seedbank estimation methods, TM and EM, although some species differed. While EROMC and ORYSA were found by the TM method, they were not evident using the EM method (Table 3). In the case of EM, the two species MALPA (three plants) and POROL (two plants) appeared, which did not appear in TM. On the other hand, differences were observed in the total number of individuals in the seedbank between the two methods. With EM, 38,528 emerged seedlings were measured, which was significantly higher than the TM method, with 14,546 emerged seedlings.

At the species level, differences were also observed between the methods (Table 4). In species such as CYPDI, 1LEMG, *Nasturtium officinale* R.Br. (NASOF), and *Ranunculus peltatus* Schrank (RANPT), more individuals were obtained by EM, while more 1ECHG, HETRE, BERCA, and ORYSA were obtained by TM. In the case of POLMO, LEFSS, *Ranunculus sceleratus* (RANSC), and SONOL the number of emerging plants was similar. There were important differences in the frequency, abundance and contribution to the seedbank of different species (Table 4). The most frequent species was CYPDI, which was found in 88.41% of the plots by TN and 97.10% by EM (100% TM+EM, data not shown), followed by POLMO (46.38% by TM and 46.38% by EM), ECHG (50.72 and 43.48%), LEFSS (40.58 and 42.03%), and NASOF (27.54 and 34.78%). According to the EM method with which the highest total values of seeds were obtained, CYPDI had the highest abundance, which made it the greatest contributor to the seedbank (90.16%), followed by POLMO (3.28%), 1LEMG (1.35%), and NASOF (1.18%). With the TM method, the species that contributed the most to the bank was CYPDI (75.98%), followed by POLMO (8.88%), 1ECHG (3.17%), LEFSS (1.70%), and NASOF (1.63%).

**Table 4.** Absolute frequency (FA), relative frequency (F), mean abundance (AM<sup>1</sup>), and specific contribution (Cs) of the main species of the germinable rice soil seedbank obtained by two estimation methods, tray method (TM) and extraction method (EM), in the Valencia rice seedbank.

Weed Species	TM				EM			
	FA	F (%)	AM	Cs	FA	F (%)	AM	Cs
<i>Cyperus difformis</i> L.	61	88.41	45.32 ± 68.91	75.98	67	97.10	129.70 ± 228.07	90.16
<i>Polypogon monspeliensis</i> (L.) Desf.	32	46.38	10.01 ± 33.43	8.88	32	46.38	9.88 ± 19.55	3.28
<i>Echinochloa</i> sp.	35	50.72	3.29 ± 6.6	3.17	30	43.48	1.70 ± 3.67	0.53
<i>Leptochloa</i> sp.	28	40.58	2.21 ± 3.66	1.70	29	42.03	1.94 ± 3.00	0.58
<i>Nasturtium officinale</i> R.Br.	19	27.54	3.12 ± 4.54	1.63	24	34.78	4.73 ± 8.89	1.18
<i>Ranunculus sceleratus</i> L.	22	31.88	1.99 ± 2.29	1.20	18	26.09	2.22 ± 2.48	0.41
<i>Ranunculus peltatus</i> Schrank	18	26.09	0.92 ± 1.25	0.45	19	27.53	1.39 ± 2.19	0.25
<i>Sonchus oleraceus</i> L.	19	27.54	0.49 ± 0.87	0.25	19	27.54	0.62 ± 1.22	0.12
<i>Lemna</i> sp.	8	11.59	7.09 ± 7.24	1.56	17	24.64	7.65 ± 8.32	1.35
<i>Amaranthus</i> sp.	5	7.25	0.3 ± 0.47	0.04	13	18.84	0.5 ± 0.93	0.07
<i>Oryza sativa</i> L.	8	11.59	0.59 ± 1.07	0.13	-	-	-	-
<i>Bergia capensis</i> L.	11	15.94	1.25 ± 1.43	0.38	1	1.45	0.75 ± 0.50	0.01
<i>Heteranthera reniformis</i> Ruiz & Pav.	3	4.35	5.5 ± 6.2	0.45	1	1.45	0.5 ± 0.58	0.01
<i>Senecio vulgaris</i> L.	3	4.35	0.25 ± 0.45	0.02	1	1.45	0.25 ± 0.5	0.00
<i>Solanum nigrum</i> L.	1	1.45	0.25 ± 0.50	0.01	2	2.90	0.25 ± 0.46	0.01
<i>Malva parviflora</i> L.	-	-	-	-	1	1.45	0.75 ± 0.96	0.01
<i>Portulaca oleracea</i> L.	-	-	-	-	1	1.45	0.50 ± 0.58	0.01
<i>Erodium malacoides</i> (L.) L'Hér	1	1.45	0.25 ± 0.50	0.01	-	-	-	-
Other/nd	65	94.20	2.70 ± 1.54	4.17	69	100	2.84 ± 1.61	2.00

<sup>1</sup> Germinated seeds in 250 g of dry soil.

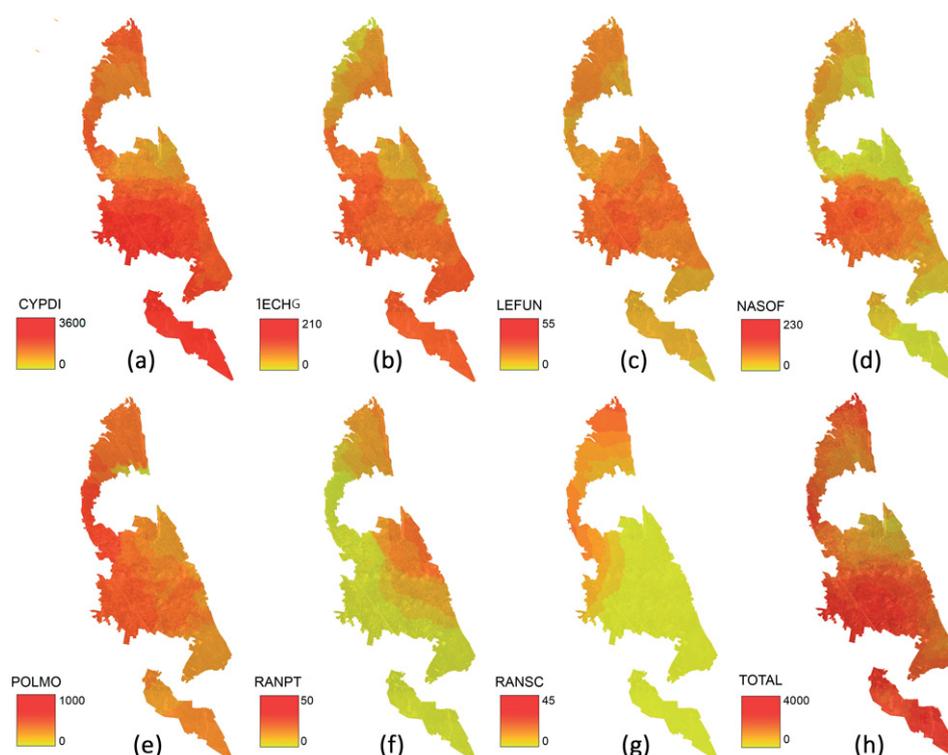
A previous analysis of the effect of winter flood level on the seedbank showed that there was no difference between flood levels 1 and 2, so the data from these two levels were pooled to analyse the results. With this, statistically significant inverse correlations were obtained between the level of the winter flood and the seedbank, with a decrease in the incidence of seeds with more water in the plots during the winter flood. Furthermore, differences in the influence of winter flood on the seedbank were found between species (Table 5). A significant inverse relationship was found between winter flooding and the seedbank in the case of CYPDI, POLMO, and 1ECHG. In RANPT, the degree of flooding had a positive effect, increasing the seedbank with more water. However, in some of the paddy species, such as LEFSS and RANSC, the degree of flooding during the winter period did not influence the seedbank.

**Table 5.** Effect of the winter flooding level on the total number of germinable seeds and the number of each species in the rice soil seedbank using a concurrent regression model.

Variable	A	CYPDI	1ECHG	POLMO	LEFSS	NASOF	RANSC	RANPT	SONOL	1LEMG
Constant	1.5202 *	1.7275 *	−0.1817	−0.2349	0.2432 *	−0.16402	−0.07917	0.25409 *	−0.00644	0.253573
Flooding level	1.0476 *	0.9334 *	0.3378*	0.4187 *	0.0399	0.2660 *	0.1797 *	−0.0573 *	0.0498 *	0.0955
F	283.63	76.43	42.78	73.92	1.70	62.88	48.31	8,72	13.61	2.37
R <sup>2</sup>	0.34	0.22	0.14	0.12	0.00	0.10	0.08	0.02	0.02	0.00
Standard error	1.2754	1.5480	0.7487	0.9985	0.6263	0.6879	0.5301	0.3976	0.2749	0.8933

(A = total soil seedbank; CYPDI = *Cyperus difformis*; 1ECHG = *Echinochloa complex*; POLMO = *Polypogon monspeliensis*; LEFSS = *Leptochloa fusca* subspecies; NASOF = *Nasturtium officinale*; RANSC = *Ranunculus sceleratus*; RANPT = *Ranunculus peltatus*; SONOL = *Sonchus oleraceus*; 1LEMG = *Lemna* sp.). \* Indicates significant effect  $p$ -value < 0.05.

Significant differences were obtained in the total germinable seedbank between plots, in addition to presenting spatial differences in distribution between the different species (Figure 4).



**Figure 4.** Cartographic representation of the spatial distribution of weed species in the study area: (a) *Cyperus difformis*; (b) *Echinochloa complex*; (c) *Leptochloa fusca*; (d) *Nasturtium officinale*; (e) *Polypogon monspeliensis*; (f) *Ranunculus peltatus*; (g) *Ranunculus sceleratus*; (h) all species.

#### 4. Discussion

The species found in the paddy seedbank of Valencia corresponded mainly to species developed in typical environmental conditions of the Mediterranean rice fields. In particular, weed species were associated with rice crops. However, more species typical of other agroecosystems have been identified in paddy fields. Something similar was obtained in the rice fields of the Camargue (France) [13] where 111 species were identified, and after the rice harvest it was detected that 82% of the weed seeds found in the soil belonged to three of the most important rice weeds. Sixty-two species of aquatic vascular plants were identified in rice crop produced in the Central Valley of California in flooded conditions [12] and several were widely distributed and well-documented weeds associated with rice. Twenty taxa were identified in the seedbank of rice fields in Malaysia [39], which were the main

weeds of the crop. Thirty-three taxa were identified in the rice paddies of Bangladesh [4], whereas twenty taxa were identified in the seedbank of wetlands in Maryland (USA) [19]. Some species, such as *Leersia oryzoides* (L.) Sw. y *Thypha latifolia* (L.), also developed in the rice fields. In the current study, 18 taxa were identified. Among the important weeds found in the seedbank of the Valencian area, weed species with high thermal requirements such as 1ECHG, CYPDI, LEFSS, HETRE, and ORYSA, whose development coincides with the rice cycle as well as other less problematic rice weeds such as BERCA or 1LEMG, were found. Species with lower thermal requirements were also found, such as POLMO, which develops in autumn–winter and whose cycle lasts until late spring, interfering with rice cultivation. NASOF, RANSC, and RANPT were also found, whose cycles do not coincide with the rice cycle. The rest of the species found in the seedbank are typical weeds of horticultural fields, both in autumn and winter (SENVU, EROMC, and MALPA) and in the spring–summer period (1AMAG, SOLNI, and POROL). These species do not develop in flooded areas, but they are frequent and abundant outside the rice paddies in orchards, field margins, and alongside roads in the area because there are horticultural and citrus fields adjacent to the rice fields of Albufera de Valencia.

In reference to the two methods used in the estimation of the seedbank (TM and EM), a similar number of species were found by both methods (15 and 16, respectively), coinciding in more than 72% of the species found (13 taxa) and differing in just five species. Thus, of the important weeds of rice, HETRE, EROMC, and ORYSA were identified by the TM method, but not by EM. Significant differences were also found between TM and EM in estimating the total number of viable seeds at the species level. The seedbank estimated with EM was 2.6 times higher than that with TM. These differences between the two methods may be mainly due to soil disaggregation and seed size. Seedbank size differences have been described in seedbank estimation methods by other authors, such as [3], who compared three estimation methods in Ohio (USA) and obtained lower estimates with TM than with other methods. Ref. [39] obtained four times more seeds using extractive methods than with direct methods similar to TM. Ref. [34] estimated the seedbank of arable crops using direct methods such as TM and extractive methods. They obtained similar estimates using both methods for grass species, but higher values for broadleaf weeds with extractive methods.

In extractive methods such as EM, soil aggregates are chemically disaggregated or dispersed, which favours the release of smaller seeds and facilitates the germination of some species such as CYPDI and 1LEMG and NASOF, whose size is less than or equal to 1 mm [35]. In the EM method, samples were passed through a 4 mm light sieve, and therefore, it is possible that some larger seeds were retained. This can be the case in 1ECHG and ORYSA seeds, or where there are structures such as edges to the seeds, as occurs in some biotypes of 1ECHG, which cause some seeds to be trapped in the sieve together with soil particles or thicker organic remains. This fact would have caused an underestimation by EM in the number of seeds of these species within the seedbank. The fact that HETRE, which has very small seeds (< 1 mm), was not detected by EM but was detected by TM, could be due to the fact that it has a lower abundance compared to the other two species and seeds may have been lost in the extraction process. Ref. [39] described something similar for species with very small seeds.

*Cyperus difformis* was the most frequent (100%) and abundant species and the one that contributed the most to the seedbank of the Valencian paddies. CYPDI appears in most seedbanks of other paddy areas around the world [4,36,38]. Seed production and its viability over time are factors of great importance in the composition of the seedbank [8], with species that produce small seeds generally being more abundant.

The frequency and abundance of species in the rice paddies of the area were influenced by the seedbank estimation obtained by the EM or TM method. More CYPDI, 1LEMG, NASOF, and RANPT seeds, and a better estimation using EM, indicated that EM was a better estimator than TM for this species. Rice weeds that did not appear with EM were detected with TM, and vice versa. This may indicate differences in estimation between

the two methods at the species level, and EM or TM may be more suitable depending on the species. Thus, for the estimation of the most important paddy seedbank species, better estimates for CYPDI are obtained by EM, while for 1ECHG, ORYSA, and HETRE, the TM method is more appropriate. Contrarily, for other important species such as LEFSS or POLMO, both methods provide similar results.

The sampling indicated that frequent species were those that appear as rice weeds (CYPDI, 1ECHG, and LEFSS). In many cases, they escape the control measures that are exerted in paddy fields from the area. Moreover, other species that occur frequently are those that develop their cycle outside of the growing season or those that are not subject to any control measures, such as POLMO, NASOF, 1LEMG, RANSC, and RANPT. The high frequency of SONOL could be because this species is very frequent and abundant in the area and its seeds have pappus, which facilitates their dispersion by the wind, so they could reach the rice fields from more distant areas to become part of the seedbank. On the other hand, in fields that are not flooded during the winter period, SONOL seeds can germinate and complete their cycle by producing seeds before paddy preparation (which is carried out in early spring to prepare for planting rice), which can increase their number in the seedbank. The same can be said for SENVU, although the frequency and abundance of this species was lower than that of SONOL.

In the composition of the seedbank at the beginning of spring, the species with the highest abundance and contribution corresponded to species that produce a large quantity of very small seeds such as CYPDI, POLMO, 1LEMG, and NASOF. LEFSS and HETRE are also very abundant species with small seeds that are important as rice weeds. Another abundant species, 1ECHG, has seeds larger than 2.8 mm [37]. From the frequency and abundance, the species with the greatest contribution to the seedbank of the rice paddies in the area was determined. The outcomes revealed seven species, CYPDI, POLMO, 1LEMG, 1ECHG, NASOF, LEFSS, and RANSC, of which three (1ECHG, LEFSS, and CYPDI) are rice weeds with the greatest control problems in the area. These are considered the most problematic weeds worldwide [38]. Poor control and greater prolificacy and longevity of these species may explain their presence and importance in the seedbank of the Mediterranean rice paddies. Similar results have been observed in studies of other rice areas, where the most abundant plants are actually weeds. These are CYPDI, LEFSS, wild rice, and 1ECHG [4,13,36]. The other four species are highly prolific. NASOF and RANSC develop their cycles in the rice paddies during the time when there is no rice cultivation, completing their cycle with a large amount of seed. The same fact occurs with POLMO, which overlaps at the beginning of rice cultivation.

The total number of seeds in the spring seedbank ( $A_1$ ) significantly decreases with winter flooding (Table 5). This is well observed in the distribution maps (Figure 4). This fact is mainly due to the contribution of CYPDI, which is the species with the highest contribution to the seedbank. Furthermore, CYPDI is the species with the greatest influence of winter flooding on the seedbank abundance (Table 5). The abundance of CYPDI decreased with proximity to the lagoon or the coast (Figure 4a).

In other species, the depressing effect of the floods on the seedbank has also been observed in POLMO, NASOF, and 1ECHG, but there are other species in which this effect does not occur and there are even species that are favoured by the degree of flood (Table 5). This is the case for RANPT, which is an aquatic plant whose development is favoured by flooding and increases in the seedbank of flooded fields (Figure 4f). Ref. [17] found a weed suppression in the second winter-flooding period in a three-year experience of developed rice fields in Japan, but some species such as *Echinochloa oryzoides* increased with the winter flooding.

The effect of flooding on the composition and abundance of the seedbank could be due to the direct effect on the viability of the seeds and the indirect effects of soil and water characteristics. Flooding causes changes in the composition and reactions of the soil, such as variations in the content of organic matter and salinity levels, which can affect the development of different plant species and, therefore, the seedbank. For example, salinity

may favour some species more adapted to saline conditions such as LEFSS [8,10] or depress the development of certain species.

Another effect associated with winter flooding is the loss of seeds due to waterfowl feeding. Losses of 69% have been reported in rice fields of La Camargue [13]. In that area a greater nocturnal activity of waterfowl was observed feeding in the flooded paddy fields. In the same trend a great reduction in the seedbank has been found throughout the winter period in flooded fields, due to the waterfowl feeding in wetlands and rice fields in the United States [14,16]. However, in this study there were no differences in seeds abundance between flooded and non-flooded fields throughout the winter period.

In addition to the effect of winter flooding, other factors influence the seedbank. In this work, it was observed that there are significant differences in the total number of seeds (Aij) between plots, which may be due to aspects as important as the operations and management carried out by farmers (soil preparation, cultivated variety, weed management, and herbicides used). Differences in the spatial distribution of different species were also found, which could be due not only to the effect of winter flooding but also to other listed factors, such as soil texture and soil composition.

The origin and management of irrigation water and the movement of seeds and propagules can also influence the seedbank. The distribution of RANSC indicates that the origin of the water (northwestern area of the Turia River and the southern area of the Júcar River) may be one of the reasons for its special distribution (Figure 4g). The area is divided into independent irrigation sectors, with water of different origins and qualities, which are managed by irrigation communities that control the moments and times when water is introduced or removed from the fields. The major methods by which rice weed seeds are dispersed are by water, by birds, in soil-by-soil machinery, and as contaminants of rice seeds [18]. With the movements of the water, seeds and propagules of different species can be moved more or less easily, which can influence the flora and the seedbank composition.

The distribution maps reflect the differences between species. CYPDI and POLMO, which are the two species with the greatest contribution to the seedbank, were widely distributed throughout the rice area (Figure 4a,e), although with lower values in the lower areas near the lagoon and the sea. These are the areas with the highest levels of waterlogging during winter flooding, which, as seen in the results, exerts a depressing effect on the seedbank of these species. The species 1ECHG was also widely distributed, although there were differences between areas. Although different species of 1ECHG were found in the area, which may vary in their cycle and adaptation to the environment, lower abundance was detected in the areas with the highest flood grade, but there were also areas with the least abundance that did not flood during the winter flood, as occurred in the plots located further north. These differences could be because it was one of the areas that was planted the earliest, with water supplies from the northern channels that had not passed through other rice lands, so the carryover of seeds may have been less. Furthermore, this area was the one that could be most easily drained to carry out herbicide treatments at the right time. This situation explains the lower presence of 1ECHG in the seedbank in these fields. It can also be influenced by the fact that areas with less 1ECHG appear to have the highest soil salinity. LEFSS was also widely distributed in the seedbank throughout the Valencian rice zone. Although the results indicate that the degree of flooding did not significantly affect the amount of seeds of this species, these results could be misleading. Two subspecies, LEFFA and LEFUN, are present in the area, with spatial distribution differences [10]. It is known that water modifies the development and adaptation of LEFSS [8,11], but their distributions may not be distinguishable. This fact could not be verified with the methodology used. Finally, in RANSC, no significant effects of winter flooding on the seedbank were obtained.

## 5. Conclusions

In this study, satellite images were used to define the flood levels of rice fields in a Mediterranean wetland during winter flooding, to study the effect of the grade of winter flooding on the spring seedbank of paddy fields.

The species that contributed most to the paddy seedbank was *Cyperus difformis* L., an important rice weed in the Mediterranean area. *Echinochloa* sp. and *Leptochloa fusca* subspecies also contributed significantly to the seedbank. Other species that develop in rice fields and interfere little or do not interfere with the rice cultivation cycle are *Lemna* sp., *Polypogon monspeliensis* (L.) Desf., and *Nasturtium officinale* R. Br. However, they clearly contributed to the seedbank in the paddy fields of Valencia.

Distribution maps of different species of weeds in the rice paddies of the area were constructed. The winter flooding had a depressing effect on the total abundance of the paddy fields seedbank. The winter flooding reduced the abundance of species such as *Cyperus difformis* L. and *Polypogon monspeliensis* (L.) Desf, which were the species that contributed the most to the seedbank. Winter flooding also reduced the seedbank of *Echinochloa* sp. It also negatively influenced the abundance of *Nasturtium officinale* R. Br. and *Ranunculus sceleratus* L. *Ranunculus peltatus* Schrank increased in the seedbank in fields that were flooded in winter. In other species, such as *Lemna* sp. and *Leptochloa fusca* ssp., which is an important rice weed in this area, winter flooding did not influence its presence in the seedbank. The seedbank can serve as a basis for understanding the potential problems of paddy weeds in the area and, therefore, rice farmers can plan weed management programmes in each plot. Finally, our outcomes show that winter flooding influences the abundance and composition of the seedbank and the distribution of plant species. This study serves as a starting point to delve into aspects that should be investigated in the area. The evolution of the seedbank throughout the winter and the influence that some factors, such as flooding period, soil characteristics, and the presence of waterfowl, have on it are key aspects to monitor, not only from the point of view of the rice crop management, but also of the integral management of a natural area of such environmental importance.

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