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Green mulching of cover crops in rice: effects on weed germination and emergence

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Abstract: **Background:** Organic rice weed management can be addressed through winter cover crops (CC). In spring, rice is seeded, CC are terminated and the field is flooded. CC residues provide a mulching layer that can reduce weed emergence, but the degradation of the biomass may also reduce weed germination through the release of organic acids. **Objective:** The objective of the study is to evaluate the effect of flooding water after CC degradation on weed germination and emergence. **Methods:** The study included both field and greenhouse trials and lasted 2 years. CC were Italian ryegrass, hairy vetch and a mixture of both. In the field, water was sampled in two locations, 5 and 10 days after flooding, and used in germination tests on weedy rice (*Oryza sativa*). In the greenhouse, a simulation of the technique was performed in buckets filled with paddy field soil: some received the CC degraded water only, others had both the water and the CC mulch layer. Water was sampled after 7 days for germination trials and total weed density in the buckets was assessed. **Results:** The germination of weedy rice was not affected by the flooding water (>92.5%). Total weed density in the buckets was reduced only when the CC mulch was present. **Conclusions:** The flooding water sampled after the degradation of the cover crop biomass did not affect weedy rice germination, both in field and in greenhouse conditions. If the CC mulch is absent, the flooding water alone is not able to reduce weeds.

Keywords: *Lolium multiflorum*; *Vicia villosa*; Flooding; Organic Rice.

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1. Introduction

Rice is one of the five most cultivated cereals in the world and in 2022 about 540,000 ha were cultivated in Europe (Food and Agriculture Organization, 2025). Italy is the first European rice producer, with approximatively 210,000 ha, which corresponds to 39% of the EU rice cultivated land (Food and Agriculture Organization, 2025). The current European Union policies, such as the European Green Deal and the Farm to Fork strategy, have the objective of reducing pesticide and fertilizer use. Moreover, organic farming is promoted by the Farm to Fork strategy and the EU objective is to have 25% of agricultural land under organic farming by 2030. In 2022, organic agricultural land share was 10.5% in Europe and Italy ranked third with 18.7% of agricultural land under organic farming (European Union, 2024; Sistema di Informazione Nazionale Sull' Agricoltura Biologica, 2023). In Italy in 2023, 4.3% of the cultivated rice area was under organic farming (Ente Nazionale Risi, 2023).

The complex ecosystem of rice fields, characterized by flooded conditions and monocropping, has led to the selection of a specifically adapted weed community, making weeds one of the main challenges in rice production (Hazra et al., 2018). While in conventional farming weed management relies mainly on herbicides, in organic farming fewer techniques are available. Mechanical weeding is one of the most commonly used techniques, but its application and efficacy are sometimes affected by soil texture and structure and by high weed density (Uno et al., 2021). Cover crops may represent a viable solution to reduce weed pressure. In addition to providing multiple benefits, such as increasing carbon fixation and enhancing nutrients availability, they are known to play a key role in suppressing weeds (Teasdale et al., 2007). Weeds can be affected not only when cover crops are actively growing, but also when residues of the cover crops are left on the soil (Kruidhof et al., 2009; Ryan et al., 2011). The dead mulch has been shown to reduce weed emergence throughout the season by reducing light interception, which depends on the thickness of the mulch layer and on the architecture of the cover crop species used (Teasdale, Mohler, 2000), and through the release of allelopathic compounds that can occur during the cover crop decomposition (Khanh et al., 2005). In recent years, organic rice farmers in Italy started to use cover crops such as *Lolium multiflorum* Lam., *Vicia villosa* Roth or a mixture of the two species in the intercropping period as a weed management technique (Fogliatto et al., 2021). Cover crops are sown during the autumn, after rice harvest; in spring, rice is seeded on the standing cover crop, which is terminated right after seeding either by shredding or rolling, and then the field is flooded (Orlando et al., 2020). The presence of a dead mulch that covers the soil after cover crop termination can significantly reduce weed emergence in paddy fields (Fogliatto et al., 2021). Moreover, the anaerobic conditions of the flooded field activate the cover crop biomass degradation processes that may lead to the release of various organic acids to a different extent, depending on temperature, soil and microbial characteristics and quantity and quality of the organic matter (Rao, Mikkelsen, 1977; Himanen et al., 2012). Some of these compounds are known to affect seed germination. For example, Masserano et al. (2022) found that acetic acid can affect rice germination, sometimes accelerating it, depending on the concentration, and sometimes reducing seedling development in some rice varieties. According to Himanen et al. (2012), the growth of both cress (*Lepidium sativum* L.) and ryegrass (*Lolium multiflorum* Lam.) seedlings was affected by organic acids and, in particular, that compounds such as formic acid and acetic acid were less toxic for example than caproic acid. Besides these compounds, some other allelochemicals may also be released during the degradation processes, especially when species such as Italian ryegrass or hairy vetch are included in the cover crop mixture. In fact, Vitalini et al. (2020) found that an aqueous extract of powdered Italian ryegrass stem, at concentrations between 20 and 100% of a 100 g/L solution, significantly reduced the germination of a typical rice weed, *Echinochloa oryzoides* (Ard.) Fritsch, while rice crop (*Oryza sativa* L.) seeds were only affected at the highest concentration. Hill et al. (2006) found that aqueous extract of hairy vetch can reduce seedling development in some crops and weeds at high concentrations, but can stimulate root growth at low concentrations. Indeed, some organic acids, such as valeric and caproic acid can have a hormetic effect and stimulate the germination in some species at low concentrations (Thomas et al., 2024). Even though the effect of the application of the dead mulching technique on the emergence of rice weeds has been assessed (Fogliatto et al., 2021), little is known on the effect that the combination of the compounds released during the decomposition of the cover crop in flooded fields may have on seed germination. Their efficacy in inhibiting seedling development is thought to be related to their concentration, which can vary depending on environmental conditions and cover crop species. In particular, higher temperatures would be expected to promote rapid degradation of cover crop biomass and thus rapid increases in organic acid concentrations, which in turn could be rapidly degraded; conversely, lower temperatures could result in slower production of organic acids, which could accumulate in the flooding water (Rao, Mikkelsen, 1977).

The objective of the study was to evaluate, both in the field and under simulated conditions, the effect of the flooding water resulting from the cover crop biomass submersion and decomposition on weedy rice (*Oryza sativa* L.) germination, used as an indicator plant, and on weed emergence.

2. Material and Methods

The experiments were carried out in Northern Italy in 2017 and 2018, with both field and greenhouse trials. Greenhouse trials were carried out at the Department of Agricultural, Forest and Food Sciences of the University of Turin in Grugliasco. Field trials were conducted on two rice farms based in two different locations, Livorno Ferraris and Rovasenda, in the province of Vercelli, within the Piedmont region (northwest Italy). These trials were conducted on the same rice fields as described in a previous study by Fogliatto et al. (2021).

2.1 Field trials

In the first farm (Livorno Ferraris), the experimental fields (45.2750°N, 8.1303°E) were ploughed to a depth of 20 cm after rice harvest and harrowed before cover crops seeding on September 28, 2016, while in the second farm (Rovasenda, 45.5386°N, 8.2968°E) cover crops were sod-seeded on October 20, 2016. In each farm, three paddy fields were selected for the trial. Two of the most common cover crop species used in the area, Italian ryegrass (*Lolium multiflorum* Lam., hereafter called ryegrass) and hairy vetch (*Vicia villosa* Roth, hereafter called vetch), were broadcast seeded at a rate of 30 kg ha^{-1} and 50 kg ha^{-1}, respectively, in two separate fields; a mixture of the two species was also broadcast seeded at 40 kg ha^{-1} (60% ryegrass, 40% vetch) in a third field.

In 2017, rice was seeded on May 13 at both sites; cover crops were terminated the following day and the fields were flooded after three days. Water was sampled twice during the growing season, 5 and 10 days after flooding (DAF) in both fields. However, at 10 DAF in Livorno Ferraris the field with the mixture had already been drained to help rice rooting, therefore the water could not be sampled. In both farms and in both years, the rice variety "Sant'Andrea" was broadcast seeded at a rate of 190 kg ha^{-1}.

Cover crops were seeded again in autumn 2017, following the same scheme and rates as the previous year. In Livorno Ferraris, cover crops were seeded on October 3 in the same fields as the previous year, after ploughing and harrowing. In Rovasenda three different paddy fields (45.5386°N, 8.2977°E) were selected in order to perform crop rotation as required by the organic farming regulation; all fields were ploughed before seeding the cover crops on October 9. Rice seeding, cover crop termination and field flooding were all carried out on April 30 in Rovasenda. In Livorno Ferraris rice was seeded on May 9, cover crops were rolled and crimped on May 13 and all fields were flooded immediately afterwards. Water was sampled twice in both fields, at 5 and 10 DAF.

Cover crop biomass at termination for both years were determined as described in Fogliatto et al. (2021); between the two years, values ranged between 1.2 and 5.6 Mg ha^{-1}

for ryegrass, 0.7 and 2 Mg ha^{-1} for vetch, 0.8 and 5.2 Mg ha^{-1} for the mixture.

In order to evaluate the influence of cover crop degradation in flooded conditions on weed germination, water sampled throughout the growing season was used for germination tests on weedy rice, being globally one of the main weed species in rice (Kraehmer et al., 2016). Twenty seeds were placed in 9 cm Petri dishes with filter paper soaked with 5 ml of flooding water. Petri dishes were sealed with Parafilm and incubated in a growth chamber at 25 °C for 15 days. Three replicates were made for each water sample, distinguished by the location (Rovasenda and Livorno Ferraris), the treatment (ryegrass, vetch and the mixture) and the sampling date. In addition, a control treatment was included for comparison using deionized water. The germination rate was assessed every day until the end of the germination trial.

2.2 Greenhouse trials

A greenhouse trial was conducted in parallel with field trials, both in 2017 and in 2018. In 2017, a wooden structure was built and divided into three independent sections of one square meter each; the bottom of each section was covered with a waterproof plastic tarp and filled with 10 cm of soil sampled in the Livorno Ferraris rice farm. On March 13, each section was seeded with a different cover crop, respectively ryegrass, vetch and a mixture of the two species, doubling the rates used in the field trial to ensure adequate coverage and biomass production. In addition, 21 buckets with a diameter of 0.3 m were arranged near the three sections and each bucket was filled with approximatively 0.016 m^3 of the same paddy field soil used in the wooden structure, in order to have a typical rice field seedbank. In particular, twelve buckets were left with bare soil, while nine buckets (three for each treatment) were seeded with the same cover crops used in the wooden structure using the same seeding rates. On May 17, cover crops were cut at the base both in the wooden structure and in the buckets. Cover crop shoots resulting from the buckets were shredded by cutting them into pieces of 5 cm length and the biomass was then redistributed equally among the three buckets of a same treatment. In the buckets with ryegrass and the mixture, the amount of fresh biomass was 250 g for each, while in the buckets with vetch the biomass was 150 g, corresponding respectively to 4.0 Mg ha^{-1} and 2.9 Mg ha^{-1} of dry matter. Additionally, any weeds that emerged from the buckets with bare soil were removed. A 7 cm layer of tap water was added to each section of the wooden structure to simulate the flooded conditions. In each section with the degrading cover crop, a pumping system was installed to allow the flooding water to circulate continuously to three buckets with bare soil for each cover crop species (ryegrass, vetch and the mixture of the two species). Also the buckets with the cover crop biomass were flooded with tap water, as well as three other buckets that were left with bare soil as a control treatment. Treatments performed with water sampled in the buckets that received the water from one of the sections were identified with the cover crop species, R for ryegrass, V for vetch and M for mixture, and the letter W, to indicate the presence of the flooding water only. Treatments performed with water sampled in the buckets that had the flooding water over the shredded cover crop to simulate the field conditions were identified with the cover crop species and the letters WM, indicating the presence of both the flooding water (W) and the mulch layer (M) in the bucket (Figure 1).

The objective of this setup was to test the effect on weed emergence by flooding water only, without interference from the mulch effect of the cover crop biomass.

After 7 days of flooding, 100 ml of water were sampled from each bucket containing the shredded cover crop, as well as from the buckets containing flooding water coming from the relative section. The water was then used to soak filter paper in Petri dishes in order to set up a germination test on weedy rice, with 20 seeds for each replicate, as described in the field trial section. The germinated seeds were recorded every day for 15 days. On May 31, water was removed from both the buckets and the wooden structure in order to simulate the drainage that is usually performed to promote rice rooting, during which weed emergence

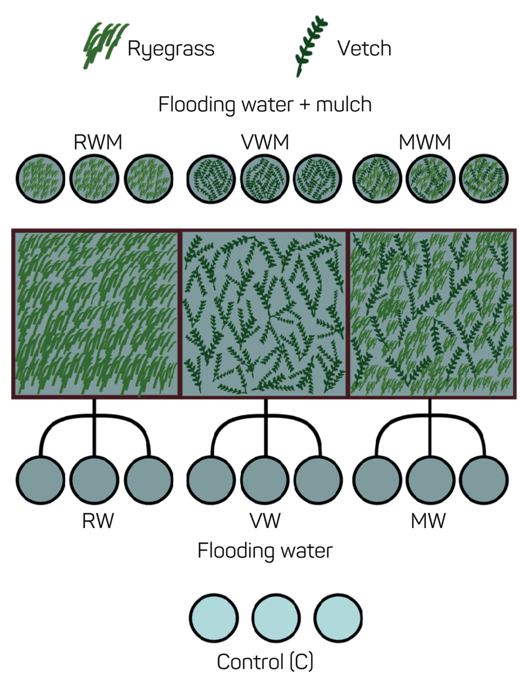


Figure 1 - Experimental setup of the greenhouse experiment. The treatments performed in the buckets are identified with the cover crop species as the first letter (R = ryegrass (*Lolium multiflorum* Lam.), V = vetch (*Vicia villosa Roth*), M = mixture of the two species) followed by W when the buckets received the water from the section with the degrading cover crop or WM when tap water was added to the buckets with the shredded cover crop; C = control with tap water

occurs. On June 16, immediately before restoring the 7 cm layer of water, weeds were counted and identified.

In 2018 the experiment was repeated using the same arrangement. On March 26 a tillage was simulated in the first 10 cm of soil and cover crops were seeded at the same rates of the previous year. On June 5 cover crops were cut, and the same amount of biomass used the previous year was distributed in the buckets; the amount of biomass was then referred to a 1 m^2 surface in order to get the same quantity of cover crop mulch in the wooden structure. After this operation, the three sections were flooded and the water pumping system was activated the same day. Water was sampled only once, on June 12 (7 DAF), in order to carry out the germination test with the same method of the previous year; germination rate was noted every day for 15 days. On July 3, weeds were counted and determined in all buckets.

2.3 Statistical analysis

Statistical analyses were carried out with R version 4.3.2 and RStudio version 2023.12.1.402 (R Core Team, 2025). Total germination of weedy rice was analysed by fitting a linear mixed effect model (*lme*) using NLME package (Pinheiro, Bates, 2025). For both trials, normality of data and homogeneity of variances were tested using the Shapiro-Wilk and Levene tests, respectively. Data were normally distributed and whenever the assumption of homogeneity of variances was violated, the *lme* model parameters were adjusted accordingly in order to account for heteroscedasticity of variances.

For the field trial, the model was fitted with the location nested in the year as a random effect and both treatment and sampling date as fixed effects. For the greenhouse trial, the model was fitted with the year as a random effect and the treatment as a fixed effect. Subsequently the untreated control was removed from the dataset and the model was fitted with the cover crop species and the presence or absence of the mulch in the buckets as fixed factors; the three buckets used for each treatment nested in the year were considered as random factors. Differences between treatments were considered significant at $p<0.05$ and means were separated with Tukey's post-hoc test.

Moreover, a three-parameters log-logistic regression model was fitted to cumulated seed germination:

$$Y = \frac{d}{1 + \exp\left[b(\log(x)\text{-}\log(e))\right]}$$

where Y is the cumulated germination, x is the time expressed in days and d, b and e are the parameters of the equation, respectively the upper limit, the relative slope and the point of inflection.

The fitted model was obtained using the *ED* function of the DRC package (Ritz et al., 2015). The model was used to calculate the germination speed, expressed as the time required by seeds to reach 50% of germination (T_{50}). Pairwise comparisons on T_{50} values were performed between the treatments for each location and sampling date through the following equation:

$$SI = \frac{T_{50}\,(A)}{T_{50}\,(B)}$$

where SI is the Sensitivity Index and A and B represent two different treatments (Ritz et al., 2006). In order to compare the T_{50} values between fitted time to event curves, the significance of SI ($p<0.05$) was obtained through the *EDcomp* function of the DRC package, that tests against the hypothesis that SI values are not dissimilar to 1. If SI > 1, the germination of 50% of the seeds is faster with the treatment B, while with SI < 1 the germination of 50% of the seeds is faster with the treatment A. The analysis was performed separately for each year, location and sampling date.

The total weed density in the buckets of the greenhouse trial was fitted to a linear mixed effect model, with year as a random factor and the treatment as a fixed factor. The control was then removed from the dataset and the model was fitted with the cover crop species and the presence or absence of the mulch as fixed factors. Differences between treatments were considered significant at $p<0.05$ and means were separated with Tukey's post-hoc test.

3. Results and discussions

3.1 Field trials

3.1.1 *Total germination*

The data obtained from the germination trial performed with flooding water sampled throughout the 2017 and 2018 growing seasons in both Livorno Ferraris and Rovasenda showed a high total germination rate of weedy rice, with values exceeding 98% in all the treatments (Table 1). Neither the treatment nor the sampling day showed an influence on the total seed germination rate, with no significant differences among treatments.

Even though the flooding water tested was not analysed for its organic acids content, it could be hypothesized that the absence of a reduction in seed germination may be attributable to two potential factors; first, the degradation of the cover crop biomass may not have led to the release of compounds with the potential to inhibit the development of weedy rice. Second, the concentration of these compounds was not sufficiently high to affect seed germination. In fact, the release of some compounds following the cover crop decomposition is linked to the amount of biomass, to the temperature and to soil properties (Tsutsuki, Ponnamperuma, 1987).

3.1.2 *Germination speed*

In 2017 in Livorno Ferraris, at 5 DAF, the weedy rice T_{50} values ranged from 2.98 days of the ryegrass treatment to 3.11 days of vetch (Table 2); pairwise comparisons through SI showed significant differences between the vetch treatment and, in decreasing order of significance, the ryegrass treatment, the control and the mixture treatment (Table 3). Despite the minimal difference in terms of T_{50}, the vetch treatment seemed to slow down seed germination. However, at 10 DAF the ryegrass treatment had the lowest T_{50} (2.27 days), while the highest T_{50} (2.67 days) was detected for the control. Significant differences were observed between ryegrass and both the control and the vetch treatment, as indicated by SI. In contrast to the previous sampling date, ryegrass accelerated the germination of weedy rice compared to the control. In 2018, weedy rice seeds treated with water coming from the ryegrass field at 5 DAF showed the shorter T_{50} (4.58 days), while the control had the longer value (4.94 days). Pairwise comparisons showed some significant differences between the control, that had the slowest germination, and all the other treatments, in decreasing order of significance ryegrass, vetch and mixture. SI also highlighted significant differences between the ryegrass and the mixture treatment, the latter having the longer germination time. At 10 DAF, T_{50} values ranged between 3.21 (ryegrass) and 3.41 days (control); the control germination time was significantly higher compared to all other treatments (ryegrass, mixture and vetch in decreasing order of significance).

Table 1 - The effect of treatment and sampling date on total germination of *Oryza sativa* L. (weedy rice) seeds treated with flooding water. Estimated marginal means of total germination are reported in the table with relative standard error in brackets

Treatment	Germination (%)
Control	98.29 [0.95]
Ryegrass	98.43 [0.90]
Vetch	98.29 [0.90]
Mixture	98.56 [0.91]
Treatment	p = 0.983
DAF	p = 0.820
Treatment ´ DAF	p = 0.752

Control: tap water, Ryegrass = *Lolium multiflorum* Lam., Vetch: *Vicia villosa* Roth, Mixture: *L. multiflorum* Lam. + *V. villosa* Roth

Table 2 - Time required by *Oryza sativa* L. (weedy rice) seeds to reach 50% of germination after treatment (T_{50}) with water sampled at Livorno Ferraris and Rovasenda at 7 and 10 DAF (days after flooding) in 2017 and at 5 and 10 DAF in 2018. T_{50} was estimated by the three-parameters log-logistic regression model. Values in brackets are the standard error of the mean

Date	Treatment	T_{50} (days)	
		Livorno Ferraris	Rovasenda
2017			
5 DAF	Control	3.01 [0.03]	2.97 [0.03]
	Ryegrass	2.98 [0.02]	2.97 [0.03]
	Vetch	3.11 [0.02]	2.97 [0.03]
	Mixture	3.04 [0.02]	2.90 [0.03]
10 DAF	Control	2.67 [0.09]	2.96 [0.03]
	Ryegrass	2.27 [0.04]	2.90 [0.05]
	Vetch	2.57 [0.04]	3.00 [0.03]
	Mixture	-	2.94 [0.03]
2018			
5 DAF	Control	4.94 [0.08]	2.72 [0.05]
	Ryegrass	4.58 [0.05]	2.70 [0.03]
	Vetch	4.64 [0.05]	2.77 [0.05]
	Mixture	4.75 [0.05]	2.71 [0.07]
10 DAF	Control	3.41 [0.04]	5.97 [0.03]
	Ryegrass	3.21 [0.03]	6.02 [0.02]
	Vetch	3.28 [0.03]	6.06 [0.02]
	Mixture	3.23 [0.03]	5.81 [0.03]

Control: tap water, Ryegrass = *Lolium multiflorum* Lam., Vetch: *Vicia villosa* Roth, Mixture: *L. multiflorum* Lam. + *V. villosa* Roth

In Rovasenda in 2017, T_{50} values of weedy rice seeds were quite similar among treatments and ranged respectively between 2.90 days (mixture) and 2.97 days (control, ryegrass and vetch) at 5 DAF, and between 2.90 days (ryegrass) and 3.00 days (vetch) at 10 DAF. No significant differences were found in pairwise comparisons. In 2018, at 5 DAF weedy rice average values of T_{50} were similar and ranged between 2.70 days to 2.77 days, respectively for the ryegrass and for the vetch treatment; in fact, no significant differences were highlighted by pairwise comparisons. On the other hand, at 10 DAF, SI values showed strongly significant differences between the mixture (T_{50} = 5.81 days) and all the other treatments. Also the pairwise comparison between the control (5.97 days) and vetch (6.06 days) pointed out a significant difference between these two treatments.

Overall, the variations in the time required to reach 50% of germination were quite small, despite the statistically significant differences between some treatments. Some studies highlighted that cultivated rice germination and seedling development seem to be affected by certain compounds, such as acetic acid (Masserano et al., 2022; Neves et al., 2010), butyric acid or propionic acid (Tunes et al., 2013), that have been shown to reduce rice germination at high concentrations, while germination speed was affected even at low concentrations. In particular, Masserano et al. (2022) found that the germination of some cultivated rice varieties can be accelerated by acetic acid at low concentrations (54 mg L^{-1}), while Neves et al. (2010) tested concentrations above 1,000 mg L^{-1} and observed a decrease in the germination speed at increasing concentrations of both acetic and propionic acid. The flooding water tested in the present field conditions induced minor changes in germination speed; however, these variations were too minimal to be considered relevant from a practical point of view.

Table 3 - Sensitivity index (SI) values of pairwise comparisons of *Oryza sativa* L. (weedy rice) seeds (ratio between T_{50} of two different treatments) treated with water sampled in Livorno Ferraris and Rovasenda at 5 and 10 DAF (days after flooding) in 2017 and at 5 and 10 DAF in 2018. (ns: non-significant; * p<0.05; ** p<0.01; *** p<0.001)

Date	Compared treatments	Livorno Ferraris			Rovasenda		
		SI	p-value		SI	p-value	
		2017					
5 DAF	Control/Ryegrass	*1.01*	0.407	*ns*	1.00	0.917	ns
	Control/Vetch	*0.97*	0.003	**	1.00	0.863	ns
	Control/Mixture	*0.99*	0.461	*ns*	1.03	0.092	ns
	Vetch/Ryegrass	*1.04*	< 0.001	***	1.00	0.949	ns
	Vetch/Mixture	*1.03*	0.014	*	1.02	0.089	ns
	Ryegrass/Mixture	*0.98*	0.086	*ns*	1.02	0.101	ns
10 DAF	Control/Ryegrass	*1.18*	< 0.001	***	1.02	0.291	ns
	Control/Vetch	*1.04*	0.298	*ns*	0.99	0.326	ns
	Control/Mixture	-	-	-	1.01	0.663	ns
	Vetch/Ryegrass	*1.13*	< 0.001	***	1.04	0.084	ns
	Vetch/Mixture	-	-	-	1.02	0.161	ns
	Ryegrass/Mixture	-	-	-	0.98	0.452	ns
		2018					
5 DAF	Control/Ryegrass	*1.08*	< 0.001	***	1.01	0.722	ns
	Control/Vetch	*1.07*	0.001	**	0.98	0.497	ns
	Control/Mixture	*1.04*	0.035	*	1.01	0.876	ns
	Vetch/Ryegrass	*1.01*	0.422	ns	1.03	0.237	ns
	Vetch/Mixture	*0.98*	0.118	ns	1.02	0.489	ns
	Ryegrass/Mixture	*0.96*	0.018	*	1.00	0.920	ns
10 DAF	Control/Ryegrass	*1.06*	< 0.001	***	0.99	0.133	ns
	Control/Vetch	*1.04*	0.017	*	0.99	0.013	*
	Control/Mixture	*1.05*	0.001	**	1.03	< 0.001	***
	Vetch/Ryegrass	*1.02*	0.110	ns	1.01	0.276	ns
	Vetch/Mixture	*1.01*	0.308	ns	1.04	< 0.001	***
	Ryegrass/Mixture	*0.99*	0.530	ns	1.04	< 0.001	***

Control: tap water, Ryegrass = *Lolium multiflorum* Lam., Vetch: *Vicia villosa* Roth, Mixture: *L. multiflorum* Lam. + *V. villosa* Roth

3.2 Greenhouse trials

3.2.1 *Total germination*

The amount of cover crop biomass added to the buckets was quite similar to that obtained under field conditions. In fact, in both years, no differences among treatments were found in the germination of weedy rice (Table 4). Germination rates ranged from 97.50% in the control to 92.50% in MWM treatment.

After removing the control from the dataset, the single effect of the cover crop and the presence or absence of the mulch, as well as the interaction between the two factors, were all found to be non-significant (Table 5). These results confirmed the findings of the field study, showing both the absence of a clear effect related to cover crop species and that the potential chemical effect is still uncertain.

3.2.2 *Germination speed*

In 2017, the treatment of weedy rice seeds with water coming from the MWM treatment resulted in the

Table 4 - The effect of treatment on total germination of *Oryza sativa* L. (weedy rice) seeds treated with flooding water derived from buckets with or without the mulch. Estimated marginal means of total germination are reported in the table with relative standard error in brackets

Treatment	Germination (%)
C	97.50 [1.12]
RWM	93.33 [3.07]
VWM	95.00 [1.82]
MWM	92.50 [2.50]
RW	96.67 [2.11]
VW	95.00 [1.29]
MW	95.83 [1.54]
Treatment	p = 0.501

C: control with tap water, RWM = ryegrass (*Lolium multiflorum* Lam.) mulch + flooding water, VWM = vetch (*Vicia villosa* Roth) mulch + flooding water, MWM = mixture (*L. multiflorum* Lam. + *V. villosa* Roth) mulch + flooding water, RW = flooding water coming from the section with degrading ryegrass, VW = flooding water coming from the section with degrading vetch, MW = flooding water coming from the section with degrading mixture of the two species

Table 5 - The effect of the cover crop and of the absence or presence of the mulch, and their interaction, on *Oryza sativa* L. (weedy rice) germination

Effect	Germination (%)
Cover crop	p = 0.902
Mulch (presence/absence)	p = 0.209
Cover crop ´ mulch	p = 0.666

longest T_{50} (2.88 days) (Table 6). The shortest T_{50} was 2.54 days, and it was obtained in the VWM treatment. Pairwise comparisons of SI values (Table 7) highlighted some differences between the treatments C and MW and between the treatments VW, MW, and all the treatments with ryegrass (RWM, RW). However, the variations in T_{50} values provided by these treatments were so small that the chemical effect of flooding water on germination seems to be negligible. In fact, in 2018, no significant differences were found in pairwise comparisons; T_{50} was higher for both the treatment RW and MWM (1.72 days), while the lower value (1.58 days) was obtained with the MW treatment (data not shown).

3.2.3 *Weed density*

In the two-year greenhouse trial, differences in terms of weed density between estimated marginal means were highly significant (p<0.001). *Heteranthera reniformis* Ruiz & Pav. was the most abundant species, followed by *Echinochloa crus-galli* (L.) P.Beauv. The average weed density in the C treatment was 1,362.8 plants/m^2. The buckets with only flooding water (RW, VW and MW) were not significantly different from the control, as the density ranged from 844.1 plants/m^2 (MW) to 1,110.6 plants/m^2 (VW). In the buckets where the mulch was also present, weed density was significantly reduced compared to the control in both the ryegrass (RWM) and in the mixture (MWM) buckets, with 521.1 plants/m^2 and 205.1 plants/m^2, respectively (Figure 2). The higher efficacy of these two treatments in weed suppression may be attributable on one hand to the higher amount of biomass applied compared to the VWM treatment (580.0 plants/m^2), which was not able to significantly reduce weed density compared to the control. On the other hand, grass species mulch are generally more efficient in reducing weeds due to a slower deterioration (Ruffo, Bollero, 2003), in particular when included in mixtures (Baraibar et al., 2018). Moreover, mixtures of cover crops seem to show a higher consistency and stability in biomass production despite the seasonal variability (Khan, McVay, 2019).

Excluding the control, the single effect of the presence of the mulch was significant (p=0.043), while the effect of the cover crop species and the interaction between these two factors were not significant (Figure 3). The presence of the mulch had a suppressive effect on weed emergence

Table 6 - Time required by *Oryza sativa* L. (weedy rice) seeds to reach 50% of germination after treatment (T_{50}) with water sampled in the greenhouse at 7 DAF (days after flooding) in 2017 and in 2018. T_{50} was estimated by the three-parameters log-logistic regression model. Values in brackets are the standard error of the mean

Date	Treatment	T_{50} (days)	
		2017	2018
7 DAF	C	2.67 [0.06]	1.61 [0.14]
	RWM	2.76 [0.06]	1.65 [0.10]
	VWM	2.54 [0.05]	1.67 [0.09]
	MWM	2.88 [0.04]	1.72 [0.06]
	RW	2.60 [0.05]	1.72 [0.06]
	VW	2.63 [0.14]	1.67 [0.22]
	MW	2.79 [0.07]	1.58 [0.26]

C: control with tap water, RWM = ryegrass (*Lolium multiflorum* Lam.) mulch + flooding water, VWM = vetch (*Vicia villosa* Roth) mulch + flooding water, MWM = mixture (*L. multiflorum* Lam. + *V. villosa* Roth) mulch + flooding water, RW= flooding water coming from the section with degrading ryegrass, VW = flooding water coming from the section with degrading vetch, MW = flooding water coming from the section with degrading mixture of the two species

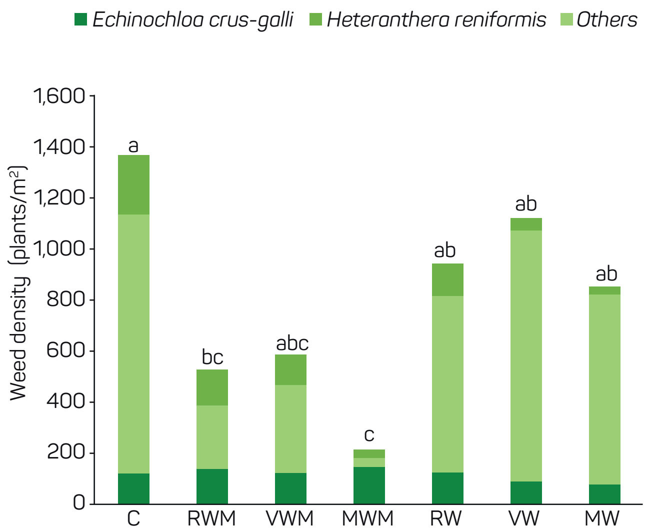
Table 7 - Sensitivity index (SI) values of pairwise comparisons of *Oryza sativa* L. (weedy rice) seeds (ratio between T_{50} of two different treatments) treated with water sampled in the greenhouse at 7 DAF (days after flooding). For 2017, only significant pairwise comparisons are listed. For 2018, no significant pairwise comparison were found (data not shown); (*p<0.05; ** p<0.01; *** p< 0.001)

Date	Compared treatments	2017		
		SI	p-value	
7 DAF	C / MWM	0.93	0.005	*
	VWM / RWM	0.92	0.003	**
	VWM / MW	0.91	0.002	**
	VWM / MWM	0.88	<0.001	***
	RW / RWM	0.94	0.038	*
	RW / MW	0.93	0.026	*
	RW / MWM	0.90	<0.001	***

C: control with tap water, RWM = ryegrass (*Lolium multiflorum* Lam.) mulch + flooding water, VWM = vetch (*Vicia villosa* Roth) mulch + flooding water, MWM = mixture (*L. multiflorum* Lam. + *V. villosa* Roth) mulch + flooding water, RW= flooding water coming from the section with degrading ryegrass, VW = flooding water coming from the section with degrading vetch, MW = flooding water coming from the section with degrading mixture of the two species

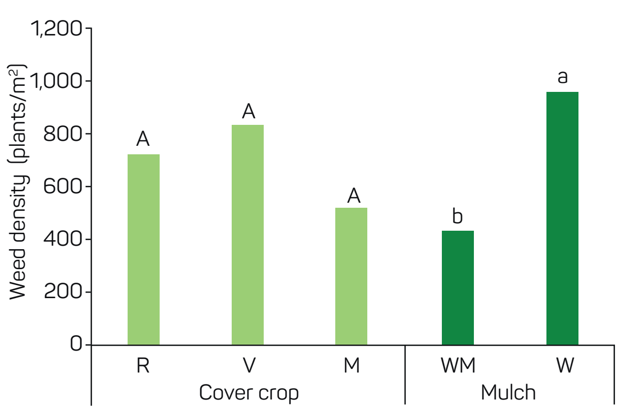
since weed density was lower where the treatment was applied compared to the buckets where only flooding water was present.

The simulation of this technique in controlled conditions has allowed to understand that the flooding water after biomass degradation in anaerobic conditions has a negligible effect on weed emergence, when considering the amount of biomass usually present in field conditions. Nonetheless, as for the mulching effect, the amount of biomass seems to play a key role in weed suppression, as the lower weed density was found where the higher amount



C: control with tap water, RWM = ryegrass (*Lolium multiflorum* Lam.) mulch + flooding water, VWM = vetch (*Vicia villosa* Roth) mulch + flooding water, MWM = mixture (*L. multiflorum* Lam. + *V. villosa* Roth) mulch + flooding water, RW= flooding water coming from the section with degrading ryegrass, VW = flooding water coming from the section with degrading vetch, MW = flooding water coming from the section with degrading mixture of the two species

Figure 2 - Total weed density in the buckets of the greenhouse trial (2 years average). Different superscript letters represent significant differences according to Tukey's post-hoc test ($p \leq 0.05$)



R = ryegrass (*Lolium multiflorum* Lam.), V = vetch (*Vicia villosa* Roth), M = mixture of *L. multiflorum* Lam. + *V. villosa* Roth, WM = tap water added to the buckets with the shredded cover crop, W = water coming from the section with the degrading cover crops

Figure 3 - Single effect of the cover crop species (average regardless the mulch treatment) and of the presence or absence of the mulch (average regardless the cover crop species). Different superscript capital letters represent significant differences between cover crops species ($p<0.05$); different superscript lowercase letters represent significant differences between the mulch treatments ($p<0.05$)

of biomass was applied; however, this relationship did not appear to be species-related in simulated conditions. On the other hand, in field conditions both soil characteristics and weather fluctuation can affect the establishment and the development of the cover crop during the intercropping season (Fogliatto et al., 2021), thereby the right choice of the cover crop single species or mixture is crucial for the success of the technique.

4. Conclusions

This study showed that the potential allelopathic effect on weed germination related to the release of organic compounds during the decomposition of the cover crop residues is still uncertain in field conditions, mainly because of the variability of the amount of biomass produced each year, that is often too low to reach effective concentrations of allelopathic compounds. This result is also confirmed by the greenhouse study, where a field-like amount of biomass was used, yet it also highlighted that weed emergence and development is reduced by a higher biomass and by the presence of a grass cover crop, both as sole species or in mixture. Nevertheless, further studies are needed to identify the organic compounds released and to assess their dynamics during the flooding period. This research will allow to determine a potentially effective concentration range of these molecules that can inhibit weed germination. Furthermore, the sensitivity to these compounds may vary between weed species, thus the effect of these molecules on other common rice weeds should be investigated.

Green mulching was developed by organic rice farmers in northern Italy as a novel strategy to combine weed control with the benefits of a cover crop grown during the fallow period. Hence, understanding the phenomena that contribute to the effectiveness of green mulching in paddy fields can help farmers to improve this technique. A significant amount of cover crop biomass is one of the key factors to ensure adequate soil coverage and, consequently, effective weed suppression. The efficacy of the sole flooding water in inhibiting weed germination still remains inconsistent and inadequate to achieve a significant reduction in weed emergence. However, the identification and the specific role of the compounds that may be released during the biomass degradation, and the impact on different species, still require further research.

Author's contributions

All authors read and agreed to the published version of the manuscript. GP, SF, and FV: conceptualization of the manuscript and development of the methodology. SF, FV, and FDP: data collection and curation. GP: data analysis. GP, SF, and FV: data interpretation. FV: funding acquisition and resources. FV: supervision. GP: writing the original draft of the manuscript. GP, SF, and FV: writing, review and editing.

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