



ORIGINAL ARTICLE

Effects of Green Mulching With Cover Crops on Rice Growth, Weed Suppression and Organic Acid Release in Flooding Water

Giulia Papandrea¹ | Silvia Fogliatto¹ | Alessandro Beltramo¹ | Barbara Moretti¹ | Zineb Bennani¹ | Martino Rabbia¹ | Maria Martin¹ | Federica Gulino² | Maurizio Aceto² | Francesco Vidotto¹

¹Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Grugliasco, Italy | ²Dipartimento per lo Sviluppo Sostenibile e la Transizione Ecologica, Università degli Studi del Piemonte Orientale, Vercelli, Italy

Correspondence: Giulia Papandrea (giulia.papandrea@unito.it)

Received: 19 February 2026 | **Revised:** 12 October 2026 | **Accepted:** 14 October 2026

Subject Editor: Husrev Mennan

Keywords: agroecology | cover crop | organic acids | organic farming | weed management

ABSTRACT

A common technique to manage weeds in organic rice in Italy involves winter cover crops (CC) that are grown in the intercropping period and terminated at rice seeding to create a mulching layer. The physical effect of the CC biomass and its degradation in flooded conditions with the release of potentially phytotoxic organic acids may impact both weeds and rice. Field trials were conducted in 2023/2024 to evaluate the impact of this technique on weed suppression and crop growth. The fields differed in the CC species, soil tillage and crop precession. Mulch-free control areas, where the CC biomass was removed, were included within each field to assess the effect of the flooding water alone. The organic acids released during biomass degradation were identified and quantified. Weed density was lower when the technique was combined with a crop precession different to rice and with ploughing prior to CC seeding (23.2 plants m⁻²) compared to fields where the preceding crop was rice and CC was seeded after minimum tillage (596.6 plants m⁻²). Overall, weed density was higher in the mulch-free control areas, while little differences were observed in rice growth compared to the rest of the field. Acetic and propionic acids were the most abundant; within the first week, acetic acid peak ranged between 158.2 and 233.9 mg L⁻¹, while propionic acid ranged between 99.8 and 167.4 mg L⁻¹. This technique can reduce weed density, but the efficacy relies upon the CC biomass, since weed control is mainly due to the presence of a mulching layer, and the agronomic practices, particularly ploughing and crop rotation.

1 | Introduction

Organic farming is an approach that combines sustainable environmental practices, the preservation of biodiversity and natural resources, and the strict limitation of the use of chemically synthesised inputs (Council Regulation (EC) No. 834/2007 2007). European Union policies promote the spread of a more sustainable food system, and the Farm to Fork strategy within the European Green Deal set the 25% of organic agricultural

land as a target to reach by 2030 (European Union 2020). Even though meeting this target in the next years is ambitious, it is crucial to support the spread of organic farming by studying sustainable alternatives to manage pests and soil fertility.

In the context of organic rice (*Oryza sativa*) cultivation, the potential contributors to yield reduction are deficiencies or unbalances in soil nutrients and severe crop–weed competition (Hazra et al. 2018). In the specific context of Italian organic

rice production, different studies highlighted that weed control remains the major challenge in order to stabilize organic rice system productivity and to reduce yield gap with conventional system (Ferrero and Vidotto 2007; Bacenetti et al. 2016). Weed control effectiveness is influenced by different factors, such as the optimisation of management techniques, the expression of the soil weed seed bank, the variety of rice, the fertility of soil, the climate and the weather conditions (Orlando et al. 2020). The effect of the latter factors are difficult to manage in the short term and are deeply related with the pedo-climatic conditions of the rice-growing area, but optimisation of management techniques for effective weed control can be achieved in several ways, including the adoption of direct mechanical methods, crop rotation, timely water management and precision-levelled fields to ensure uniform flooding depth (Ferrero et al. 2021; Uno et al. 2021). In most cases, the combination of different techniques is more effective than the implementation of a single technique (Blackshaw et al. 2001).

Recently, the agroecological practice of using green mulch from various cover crops (CC) has become the dominant technique in Italian organic rice farming. This method facilitates the incorporation of nitrogen (N) when leguminous CC are used, while also controlling weeds (Orlando et al. 2020; Fogliatto et al. 2021; Vaglia et al. 2022). The CC, for example *Lolium multiflorum* (Italian ryegrass), *Secale cereale* (rye) and *Vicia villosa* (hairy vetch), is seeded in autumn and cultivated in the intercropping period. Subsequently, a variety of techniques can be employed to terminate the CC (e.g., shredding, rolling or left standing) and rice is broadcast seeded either before or after the CC termination, depending on the farmer's requirements. Immediately after CC termination, fields are flooded.

Weed control in the green mulching technique is potentially obtained with: (i) the direct competition between CC and weeds during the CC growing period; (ii) the formation of a physical barrier on soil surface after CC termination, that hinders the growth of weeds in rice crop; (iii) the release of chemical compounds from the living or dead tissues of CC species with phytotoxic effects; (iv) the production of organic acids (such as acetic, propionic and butyric acid) derived from biomass fermentation in flooded condition (Vitalini et al. 2020; Fogliatto et al. 2021). The accumulation of these acids is due to the establishment of anaerobic conditions in flooded paddy soils and the consequent shift of microbial activity towards anaerobic metabolic pathways (Le Mer and Roger 2001). Acetic acid (AA) is the most produced organic acid from rice straw degradation (Camargo et al. 2001; Oliveira de Sousa et al. 2002): it reaches its maximum concentration within few days after the beginning of flooding (Chandrasekaran and Yoshida 1973; Bohnen et al. 2005) and then gradually declines after direct volatilisation or microbial transformation to methane (Angeles et al. 2006). The amount of AA released is affected by temperature, pH, the quality and quantity of biomass and soil management factors (Tsutsuki and Ponnampetuma 1987; Biswas et al. 2001). During the phase of accumulation, AA can damage rice seminal roots and shoots to a different extent according to both AA concentration and rice variety (Masserano et al. 2022).

In a previous study conducted by Fogliatto et al. (2021), the weed control efficacy of this technique was investigated by comparing

different CC. The authors highlighted that even though CC biomass is a crucial parameter, the ability to suppress weeds was variable depending on the field management, on the seedbank and on climatic conditions. Moreover, they hypothesized that the organic acids released during the biomass fermentation could have played a role in reducing weed germination (Fogliatto et al. 2021). Subsequently, Papandrea et al. (2025) observed in simulated paddy field conditions that the flooding water resulting from CC biomass fermentation was not able to reduce weed emergence, as weeds were mostly controlled by the presence of a CC residue mulch. On the other hand, this aspect has not yet been investigated in field conditions, nor have the specific acids released during the CC flooding period been identified.

As this technique is typically applied by organic farmers, the present field study was conducted through an on-farm approach, adhering to the farmers' habits and to their variations of this practice. The first objective of the study was thus to evaluate the efficacy of different applications of the green mulching technique in controlling weeds. Secondly, we sought to establish specific areas within each field where the CC residue mulch was absent, in order to investigate the extent to which the flooded mulch layer affects weeds and rice in comparison to areas where only the flooding water was present. The final objective was to study the dynamic behaviour of organic acids in the flooding water, by identifying and quantifying them during the CC biomass degradation process.

2 | Materials and Methods

2.1 | Site Characteristics, Experimental Design and Agronomic Management

A 2-year field trial was conducted in 2022–2023 and 2023–2024 on two organic rice farms located in north-west Italy, in the Piedmont region. Both farms apply the green mulching technique for weed control, as described in the introduction, although with some minor differences in the management practices. Farm 1 applies green mulching according to the traditional technique (T), which entails the seeding of rice on the standing CC, immediately followed by rolling the CC and flooding the field. In Farm 1, Italian ryegrass (*L. multiflorum*) was used as a CC, at a seeding rate of 50 kg ha⁻¹. Farm 2 applies both the traditional technique (with the difference that rice is seeded immediately after CC rolling) and an innovative technique (I), which involves rolling the CC, flooding the field and subsequently seeding rice in water approximately 10 days later. Therefore, in the case of the innovative technique CC has undergone some anaerobic decomposition processes prior to rice seeding; in this farm, a mixture of rye (*S. cereale*), hairy vetch (*V. villosa*) and Italian ryegrass (*L. multiflorum*) was seeded respectively at 40, 30 and 30 kg ha⁻¹. Farm 2 typically applies the innovative technique in fields where the previous crop was different from rice, for example rye (*S. cereale*). In such cases, the CC is seeded earlier, following the harvest of the previous crop and the ploughing of the field. Conversely, the traditional technique is usually applied after one rice season, therefore the CC is seeded later in the autumn, after the rice harvest and a minimum tillage operation. Seven fields were considered for the trial, with three fields selected in the first year and four in the second year; the field size ranged between 10 000 and 24 000 m².

In 2022–2023, two fields (1_T, Farm 1 and 2_T, Farm 2) were managed with the traditional technique (both with rice as a previous crop and minimum tillage), while a third field (3_I, Farm 2) was managed with the innovative technique, implying rye as a previous crop and ploughing. In 2023–2024, two fields were managed with the traditional technique: 4_T in Farm 1, following buckwheat (*Fagopyrum esculentum*) and ploughing, and 5_T in Farm 2, following rice and minimum tillage. Two more fields were managed with the innovative technique: in field 6_I (Farm 2) the technique was applied as usual, with rye as a previous crop and ploughing; in field 3_I (already considered in 2022–2023 in Farm 2), the innovative technique was applied again, but after one rice season and with minimum tillage. The treatment was repeated on the same field only in the case of the field 3_I. A diagrammatic illustration of fields layout and agronomic practices is reported in Figure 1. Field operations of all fields are summarized in Table 1 for the 2022–2023 season and in Table 2 for the 2023–2024 season.

In each field, four control areas of 4 m² each were created along the field diagonal. In these areas, the CC was cut at the base before the termination (which occurs before flooding the field) and the biomass was removed. While the effect on crop and weeds in the field is attributable to both the physical mulching effect of the rolled CC and the organic acids released in flooding water, the controls were exclusively influenced by the potential phytotoxic effect of the compounds released during the CC decomposition. Creating these areas within each field allowed to have a similar seedbank between the controls and the rest of the field. In field 3_I, that was considered for both years, different control areas were made in the 2 years (Figure 1).

In the first year of the trial, both winter and spring were characterized by low rainfall; in particular, cumulative rainfall of March and April was only 54.0mm, while both in May and in June the total precipitation was above 100mm (Figure 2). In the second year, starting from February precipitations were

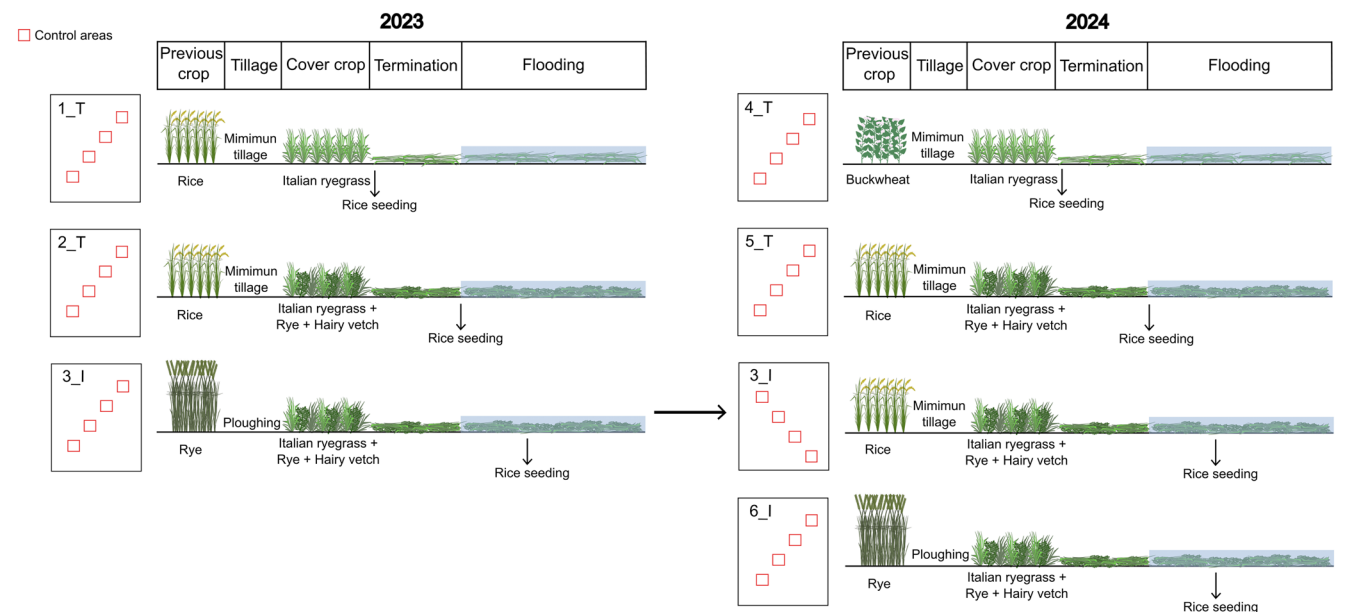


FIGURE 1 | Experimental fields layout in 2023 and 2024. Red squares represent control areas in which the cover crop was removed prior its termination. The letter T near the field number indicates the traditional technique, the letter I the innovative technique. Field 1_T and 4_T belong to Farm 1, that applies the traditional technique by seeding rice on the standing CC, terminating the CC and flooding the field immediately after. Farm 2 applies both the traditional technique (but rice is seeded immediately after CC termination) and the innovative technique, which involves terminating the CC, flooding the field and seeding rice in water approximately 10 days later. Field 3_I was considered in both years but in 2024 control areas were different from 2023. Crops: Rice (*Oryza sativa*), rye (*Secale cereale*), buckwheat (*Fagopyrum esculentum*). Cover crops: Italian ryegrass = *Lolium multiflorum*, Rye = *Secale cereale*, Hairy vetch = *Vicia villosa*.

TABLE 1 | Field operations carried out in 2022–2023 for the first year of trial.

2022–2023			
Operation	1_T (Farm 1)	2_T (Farm 2)	3_I (Farm 2)
CC seeding	3/10/2022	29/10/2022	7/10/2022
CC termination	12/04/2023	21/05/2023	5/05/2023
Rice variety and seeding rate	Dardo 240 kg ha ⁻¹	Gladio 225 kg ha ⁻¹	Gladio 225 kg ha ⁻¹
Rice seeding	12/04/2023	21/05/2023	18/05/2023
Rice harvest	14/09/2023	18/10/2023	11/10/2023

TABLE 2 | Field operations carried out in 2023–2024 for the second year of trial.

2023–2024				
Operation	3_I (Farm 2)	4_T (Farm 1)	5_T (Farm 2)	6_I (Farm 2)
CC seeding	18/10/2023	10/10/2023	8/11/2023	5/10/2023
CC termination	29/04/2024	13/05/2024	13/05/2024	29/04/2024
Rice variety and seeding rate	Lasjkk20 250 kg ha ⁻¹	Cammeo 180 kg ha ⁻¹	Lasjkk20 250 kg ha ⁻¹	Lasjkk20 250 kg ha ⁻¹
Rice seeding	16/05/2024	13/05/2024	13/05/2024	13/05/2024
Rice harvest	14/10/2024	7/10/2024	- ^a	11/10/2024

^aField 5_T was not harvested due to poor rice development.

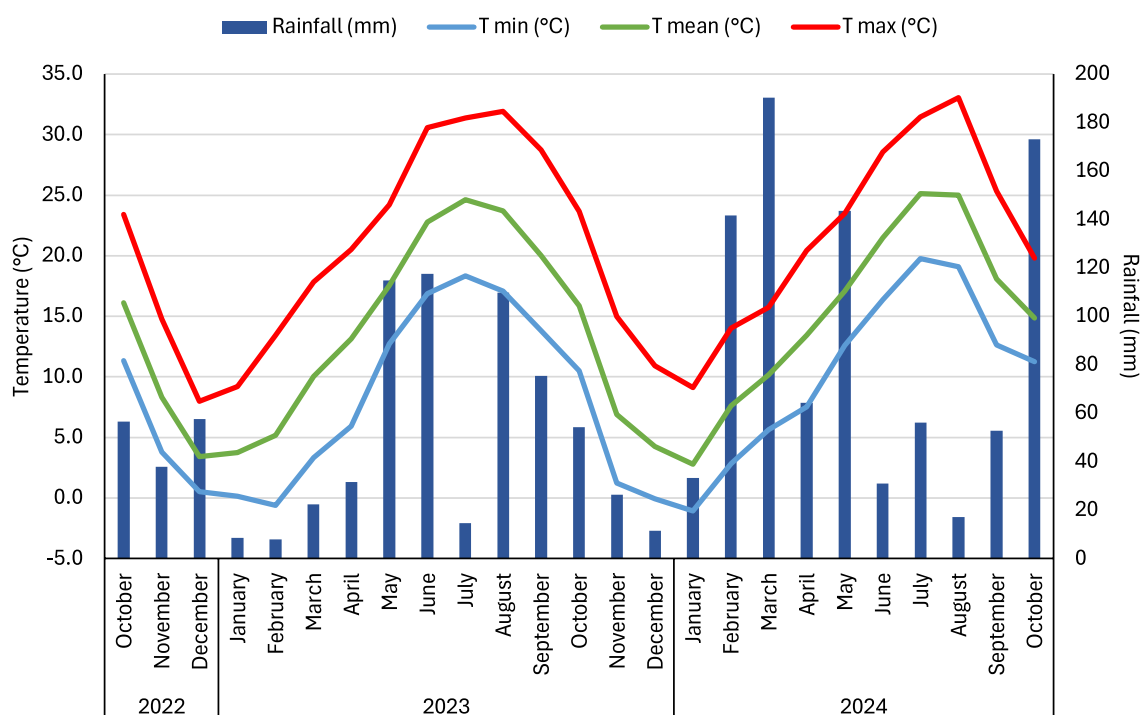


FIGURE 2 | Meteorological data retrieved from the nearest weather station (Crescentino) from October 2022 to October 2024 (Rete Agrometeorologica Regione Piemonte).

more abundant compared to the previous year, with the highest monthly rainfall recorded in March (190.2 mm). Temperatures were similar between the 2 years, with the highest average temperature recorded in July (25°C). Meteorological data were retrieved from the nearest available weather station (45.179263° N, 8.099674° E) from Regione Piemonte (Rete Agrometeorologica Regione Piemonte).

2.2 | Sampling and Chemical Analyses

In both years, CC biomass was assessed prior to termination by randomly sampling 10 quadrats of 0.25 m² in each field. Plants were cut at the base and oven dried at 80°C for 5 days. Average CC height (cm) and CC soil cover (%) were also measured. During rice growth, weed density (plants m⁻²) was assessed twice (t1: tillering, t2: stem elongation) in two quadrats of 0.25 m² per each

control area (8 quadrats in total) and in 15 quadrats of 0.25 m² in the rest of the field. At t1 and t2, rice height (cm) and SPAD index (SPAD 502Plus Konica Minolta) were also measured; at the end of flowering, both rice height and density (panicles m⁻²) were recorded. Rice was manually harvested by sampling 4 quadrats of 1 m² in the four control areas and 4 quadrats of 1 m² in the rest of the field. Rice establishment was poor in the field 5_T due to a severe weed infestation, so the crop was not harvested in this field. For each grain sample, total grain yield and thousand grain weight (TGW) were measured and adjusted at 13% moisture.

Starting from the beginning of the field flooding until the first drainage to help rice rooting, water samples were collected at 2–3-day intervals in 2023 and daily in 2024 in all fields except 4_T. This field was excluded in order to sample water in closely located fields, since 4_T was located farther from the other fields and had different soil characteristics (higher silt rate). Water samples were

filtered using a 0.20µm syringe filter and analysed through ion exclusion chromatography to assess the concentration of oxalic, citric, formic, lactic, acetic, succinic and propionic acids, which are known to be produced during biomass fermentation and/or to exhibit phytotoxic effects (Himanen et al. 2012; Meryemoglu et al. 2023). In 2024, incoming plain water was also sampled in order to exclude the presence of such organic acids in the flooding water before the beginning of the CC decomposition. The chromatographic conditions were as follows: sample loop volume: 25µL; stationary phase: Dionex IonPac ICE-AS6 9×250 mm analytical column (Thermo Fisher Scientific, Waltham, USA); mobile phase: 0.4mM heptafluorobutyric acid; eluent flow rate: 1.0mL/min; suppressor: AMMS-ICE 300; regenerant solution: 5mM tetrabutylammonium hydroxide; temperature: 20°C.

2.3 | Statistical Analysis

The statistical analysis was conducted with R version 4.5.2 (R Core Team 2025). Cover crop biomass, height and visual soil cover, as well as weed density prior to CC termination, were analysed by fitting a linear mixed-effects model (*lme*) using NLME package (Pinheiro and Bates 2025). The model was fitted with the sampling quadrats as a random factor and the field as a fixed factor. The data from the 2years were analysed separately because different fields and different rice varieties were included in the trial.

The data retrieved on weeds were analysed in two steps. First, the data from the control areas were removed from the dataset to compare the infestation level observed in the various fields and the related applied techniques. In particular, weed density was analysed by fitting a linear mixed model with the interaction between the field and the crop stage (t1 and t2) as fixed factor, and with the sampling quadrats as random factor. Means were separated with Tukey's post hoc test ($p \leq 0.05$); when the interaction was significant, means were separated by field within each crop stage. In the second step, data related to the control areas were included in the dataset, with each field and crop stage being considered separately, in order to compare weed density between the control areas and the rest of the field, highlighting the effect of flooding water only on weed density. For each assessment (t1 and t2), a two-tailed Student's *T*-test was computed.

A further comparison was made between the control areas and the rest of the field regarding rice height, SPAD index and yield parameters (rice panicle density, grain yield and thousand grain weight). Each field and each sampling date were analysed separately, and a two-tailed Student's *T*-test was computed for each assessment and parameter. Data related to the crop development were not compared among fields since rice varieties were different.

3 | Results

3.1 | Cover Crop Development in the Intercropping Period

In 2023, CC biomass was significantly higher in 2_T (613.2 g m⁻²) compared to 1_T (310.4 g m⁻²), but 3_I (553.6 g m⁻²) was not dissimilar from both (Table 3). With regard to CC

TABLE 3 | Cover crop (CC) biomass, height and visual soil cover in the CC prior termination in 2023 and 2024 among different fields.

Year	Field	CC biomass (g m ⁻²)	CC height (cm)	CC visual soil cover (%)
2023	<i>p</i>	<0.001	<0.001	<0.001
	1_T	310.4b	26.5b	77.5b
	2_T	613.2a	121.4a	97.0a
	3_I	553.6ab	100.5a	100.0a
2024	<i>p</i>	0.036	0.001	<0.001
	3_I	378.7ab	66.5b	89.0a
	4_T	272.2b	81.3ab	62.0b
	5_T	375.2ab	77.4ab	84.2a
	6_I	504.2a	84.3a	88.0a

Note: Different letters represent significant differences according to Tukey's post hoc test ($p \leq 0.05$). T= traditional technique; I= innovative technique.

height and visual soil cover, both 2_T and 3_I had significantly higher values compared to 1_T. In all three fields, the weed density prior to termination was lower than 1 plant m⁻² (data not shown). In 2024, CC biomass was significantly higher in 6_I (504.2 g m⁻²) compared to 4_T (272.2 g m⁻²), while 3_I and 5_T had similar biomass: 378.7 and 375.2 g m⁻², respectively. Furthermore, a significantly higher CC height was observed in field 6_I (84.3 cm) in comparison to 3_I (66.5 cm). The field 4_T had the highest weed density in the standing cover crop (112.0 plants m⁻²) in comparison to the other fields, which exhibited weed density levels below 15 plants m⁻² (data not shown), and the lowest CC soil cover (62.0%), which was always above 80% in the other fields.

3.2 | Weed Density

3.2.1 | Between-Field Comparison

In 2023, both weed density and weed soil cover were statistically different among fields, while neither the crop stage nor the interaction between crop stage and field was significant (Table 4). Weed density was significantly lower in field 3_I (23.5 plants m⁻²) compared to the fields that were managed with the traditional technique (83.1 plants m⁻² in 1_T and 51.1 plants m⁻² in 2_T). The weed soil cover percentage was also significantly lower in field 3_I.

In 2024, the interaction between the two factors (field and crop stage) was significant, therefore means in Table 4 were displayed by field at different crop stages. At t1, field 6_I exhibited the lowest weed density (13.3 plants m⁻²), while field 5_T had the highest infestation (658.9 plants m⁻²). Fields 3_I and 4_T showed similar weed density values (156.3 and 214.9 plants m⁻², respectively) and differed significantly from the other two fields. The lowest weed soil cover was recorded in field 6_I (5.7%), while the highest value was found in field

TABLE 4 | Effect of the field and the crop stage on weed density and weed soil cover.

		Weed density (plants m ⁻²)		Weed soil cover (%)
Factor		<i>p</i>		<i>p</i>
2023				
Field		<0.001		<0.001
Crop stage		0.927		0.102
Field × Crop stage		0.053		0.158
Field	1_T	83.1a	38.6a	
	2_T	51.1a	34.2a	
	3_I	23.5b	13.9b	
Crop stage	t1	51.5	24.7	
	t2	53.6	33.1	
		Weed density (plants m ⁻²)		Weed soil cover (%)
Factor		<i>p</i>		<i>p</i>
2024				
Field		<0.001		<0.001
Crop stage		0.196		<0.001
Field × Crop stage		0.008		<0.001
Crop stage	Field			
t1	3_I	156.3b	22.7b	
	4_T	214.9b	13.9bc	
	5_T	658.9a	54.3a	
	6_I	13.3c	5.7c	
t2	3_I	137.9b	50.9b	
	4_T	92.0bc	25.0c	
	5_T	569.6a	77.0a	
	6_I	23.2c	6.1d	

Note: Estimated marginal means are reported. Different letters represent significant differences according to Tukey's post hoc test ($p \leq 0.05$). When the interaction is significant, means are separated by field within each crop stage. T = traditional technique; I = innovative technique; t1 = tillering; t2 = stem elongation.

5_T (54.3%). At t2, weed density was significantly higher in field 5_T (596.6 plants m⁻²) compared to all other fields; field 3_I had a higher infestation compared to field 6_I (137.9 and 23.2 plants m⁻², respectively), while 4_T (92.0 plants m⁻²) did not differ significantly from these two fields. As for weed soil cover, all fields were statistically different each other; the highest value was found in field 5_T (77.0%), followed by 3_I (50.9%), 4_T (25.0%) and 6_I (6.1%).

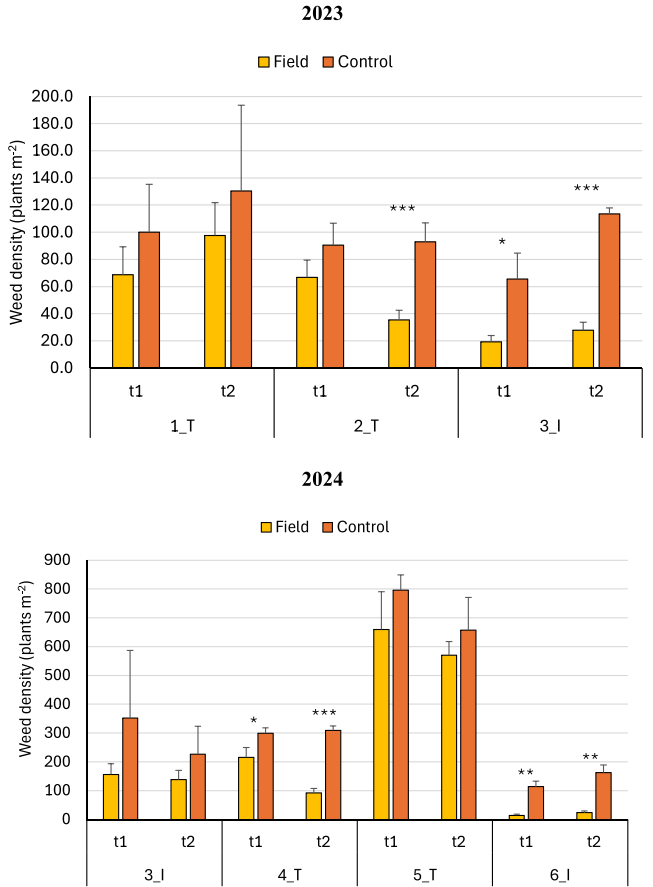


FIGURE 3 | Average weed density in the control areas with no cover crop mulching and in the rest of the field in 2023 and 2024 (* $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$). T = traditional technique, I = innovative technique, t1 = tillering, t2 = stem elongation.

3.2.2 | Within-Field Comparison

For each year of the study, statistical differences in terms of weed density between the areas in which CC was removed and the rest of the field were found in only two fields: in 2_T and 3_I in 2023 and in 4_T and 6_I in 2024 (Figure 3).

In 2023, in field 1_T no significant differences were observed between the control and the rest of the field at either crop stage. The average weed density in the first assessment (t1) was 100.0 plants m⁻² in the control areas and 68.6 plants m⁻² in the rest of the field, while at t2 weed density was slightly higher. In field 2_T, significant differences were found in the second assessment only (t2), when the average weed density was 93.0 plants m⁻² in the control and 35.5 plants m⁻² in the rest of the field. In field 3_I, weed density was significantly different between the control and the rest of the field in both assessments. At t1, the infestation inside the control areas was 65.6 plants m⁻², while in the rest of the field the weed density was 19.2 plants m⁻². At t2, the average weed density was 113.5 plants m⁻² and 27.7 plants m⁻², respectively.

In 2024, in the fields in which the traditional technique was applied, significant differences were observed in the field 4_T only. In particular, in the first assessment (t1) the average weed density was 299.5 and 214.9 plants m⁻² respectively in the control and in

the rest of the field. In 5_T, the infestation was severe, with no differences between the control and the rest of the field; the final weed density recorded in the control areas was 657.0 plants m⁻². As for the innovative technique, in field 3_I no significant differences were found, despite having at t1 an average weed density of 351.4 plants m⁻² in the control and 156.3 plants m⁻² in the rest of field. At t2, the infestation in the control areas was lower than at t1, but not dissimilar from the rest of the field. Conversely, in field 6_I at t1 the average weed density in the control areas was 113.5 plants m⁻², which was significantly higher compared to the rest of the field (13.3 plants m⁻²). Also in the second assessment, the density was significantly different between the two treatments.

3.3 | Rice Growth and Yield

In 2023, no differences in rice height were found between the control and the rest of the field, in the fields in which the traditional technique was applied (Table 5). On the other hand, in 3_I rice height was slightly but significantly higher in the field compared to the control at t1 (42.3 and 39.8 cm, respectively). Regarding SPAD index, both in 1_T and in 2_T significant differences between the field and the control were found at t1, respectively 40.5 and 31.8 in 1_T and 28.6 and 30.7 in 2_T. In 2024, significant differences were found between the control and the rest of the field in 3_I and 4_T. In 3_I at t2 rice height was significantly higher in the field than in

the control (55.7 and 50.2 cm, respectively). In 4_T, rice height was significantly higher in the rest of the field than in the control both at t1 (42.3 and 39.8 cm, respectively) and at t2 (65.6 and 52.4 cm, respectively). Concerning the SPAD index, in both fields managed with the traditional technique at t1 the field average was found to be significantly lower compared to the control, while in both fields managed with the innovative technique, at t2 the SPAD index was significantly higher in the field (37.3 in 3_I and 38.1 in 6_I) compared to the control (34.2 in 3_I and 35.0 in 6_I).

In 2023, no differences were found in the final density of the crop (Table 6). Overall, the highest density was found in the field 3_I, managed with the innovative technique, which had 355.2 panicles m⁻² in the field. The two fields where the traditional technique was applied had similar densities: 168.0 and 164.3 panicles m⁻² respectively for 1_T and 2_T in the field, and 142.5 and 138.0 panicles m⁻² in the control. Significant differences in the final grain yield were found in 1_T only, with 35% increase in the field compared to the control. In 3_I the grain yield was higher (5 Mgha⁻¹) than in 2_T (2.4 Mgha⁻¹). Thousand grain weight was similar between field and control in all fields.

In 2024, significant differences between the treatments in the crop density were only found in the fields managed with the

TABLE 5 | Average rice height and SPAD index in the control areas with no cover crop mulching and in the rest of the field in 2023 and 2024.

Field	Treatment	Height (cm)		SPAD index	
		t1	t2	t1	t2
2023					
1_T	Field	49.8	66.0	40.5***	38.5
	Control	47.8	67.0	31.8***	37.5
2_T	Field	48.9	64.2	28.6*	30.5
	Control	49.4	60.8	30.7*	31.1
3_I	Field	42.3*	60.7	32.3	33.6
	Control	39.8*	60.3	30.9	34.6
2024					
3_I	Field	27.3	55.7**	32.8	37.3***
	Control	24.0	50.2**	32.3	34.2***
4_T	Field	28.7***	65.6***	29.8**	31.1
	Control	32.2***	52.4***	32.3**	30.9
5_T	Field	24.1	53.0	29.0**	28.9
	Control	25.5	53.6	31.4**	28.6
6_I	Field	36.2	71.9	39.2	38.1**
	Control	35.7	71.1	38.3	35.0**

Note: T = traditional technique, I = innovative technique, t1 = tillering, t2 = stem elongation.
*p ≤ 0.05.
**p < 0.01.
***p < 0.001.

TABLE 6 | Average rice density, grain yield and Thousand Grain Weight (TGW) in the control areas with no cover crop mulching and in the rest of the field in 2023 and 2024.

		Density (panicles m ⁻²)	Grain yield (Mgha ⁻¹)	TGW (g)
Field	Treatment			
2023				
1_T	Field	168.0	5.4*	25.0
	Control	142.5	3.5*	25.8
2_T	Field	164.3	2.5	28.0
	Control	138.0	2.4	27.7
3_I	Field	355.2	5.1	25.5
	Control	285.5	5.0	25.0
2024				
3_I	Field	171.7	6.2	52.2
	Control	145.5	4.2	49.7
4_T	Field	270.4***	4.2	42.5**
	Control	133.5***	3.1	39.3**
5_T	Field	47.2*	_a	_a
	Control	19.5*		
6_I	Field	230.1	6.2	50.6
	Control	171.5	5.7	51.8

Note: Both grain yield and TGW are adjusted to 13% moisture. T = traditional technique, I = innovative technique.
^aField 5_T was not harvested due to poor rice development.
*p ≤ 0.05.
**p < 0.01.
***p < 0.001.

traditional technique. In 4_T and 5_T, the density was significantly higher in the field (270.4 and 47.2 panicles m^{-2} , respectively) compared to the control (133.5 and 19.5 panicles m^{-2} , respectively). Yet, the overall crop emergence in field 5_T was compromised by the high weed presence. In the fields managed with the innovative technique, the final density was on average 162.6 panicles m^{-2} in 3_I and 209.7 panicles m^{-2} in 6_I, regardless the treatment. No significant differences in final yield were found between the treatments in any field, which ranged from 3.7 to 6.2 Mg ha^{-1} . A slight reduction of thousand grain weight was found in the control areas of field 4_T, 39.3g compared to 42.5g in the rest of the field.

3.4 | Organic Acid Content

No organic acids were detected in the incoming plain water, indicating that such compounds were absent in the flooding water prior to the CC decomposition.

In 2023, in field 1_T (Figure 4) oxalic acid peaked twice during the first flooding period, on the third day (42.2 mg L^{-1}) and on the seventh day (38.1 mg L^{-1}), while acetic acid peaked on the fifth day (20.9 mg L^{-1}). Formic acid had the highest overall concentration (71.0 mg L^{-1}) that was reached after 10 days of flooding. In field 2_T, all acids were below 20 mg L^{-1} , except acetic and propionic acids on the fifth day (35.9 and 26.2 mg L^{-1} respectively). In field 3_I, on the first day of flooding acetic acid was the most abundant (96.5 mg L^{-1}), followed by lactic acid (36.1 mg L^{-1}) and oxalic acid (27.5 mg L^{-1}); the highest concentrations were exhibited by acetic and propionic acid on the fourth day (58.2 and 99.8 mg L^{-1} , respectively).

In 2024, in field 3_I four acetic acid peaks were observed: 55.7 mg L^{-1} after 3 days of flooding, 32.6 mg L^{-1} after 5 days, 45.3 mg L^{-1} after 7 days and 60.8 mg L^{-1} after 9 days. A peak of 31.3 mg L^{-1} was observed for propionic on the third day, while all the other acids were at concentrations below 10 mg L^{-1} . Also in field 5_T, acetic and propionic acid had the highest concentration; in particular, they both peaked on the seventh day of flooding with a concentration of 48.4 and 99.4 mg L^{-1} , respectively. Field 6_I exhibited an unusual behaviour, with acetic and propionic acid reaching a first peak on the fourth day (233.9 and 167.4 mg L^{-1} , respectively), followed by a decrease over the subsequent 2 days and then a gradual increase. Notably, propionic acid reached 382.6 mg L^{-1} on the twelfth day and 587.4 mg L^{-1} on the final day, the highest values ever recorded in this study.

4 | Discussion

The green mulching technique in rice spread among organic farmers in northern Italy in recent years and has been previously analysed in different studies (Orlando et al. 2020; Vitalini et al. 2020; Fogliatto et al. 2021; Vaglia et al. 2022; Papandrea et al. 2025). Nonetheless, the efficacy of this technique to control weeds depends on many factors, resulting in variability in the data acquired over the years.

Cover crop biomass is a crucial parameter in weed control (MacLaren et al. 2019) and CC mixtures generally seem to produce higher biomass levels, since different species can adapt to different environmental conditions (Khan and McVay 2019). In fact, in both years of this study, a higher biomass and a higher soil cover were observed in the fields where a CC mixture was

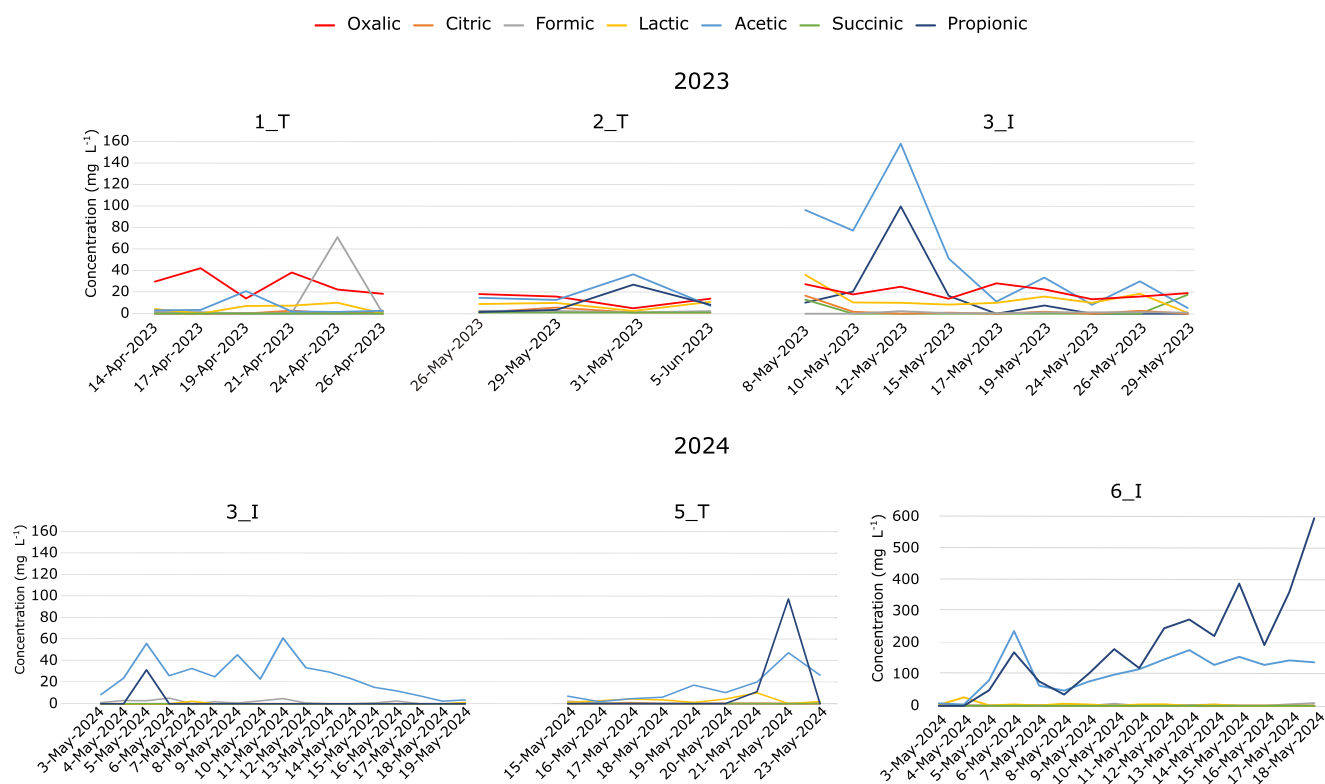


FIGURE 4 | Organic acid concentration trend in flooding water sampled from the beginning of flooding until the first drainage for rice rooting. T=traditional technique, I=innovative technique.

seeded. On the other hand, in the present study different weed densities were observed in fields in which cover crops developed similarly. For instance, in 2024 fields 3_I and 5_T had a similar CC biomass, but in the latter a severe infestation compromised the crop growth. This aspect highlights that the success of this technique is not solely dependent on CC biomass. In this study we did not focus on the species effect of the CC, which has been previously investigated (Fogliatto et al. 2021; Moretti et al. 2025), but rather on how different applications of the same agroecological approach can lead to different results, given the combination of agronomical practices that depend on the farmers' needs.

The innovative technique involves CC seeding after 1 year growing a rotational crop such as rye, whose residues are usually incorporated into the soil through ploughing. The traditional technique instead is applied after one or two rice seasons and in this case a minimum tillage is typically performed before seeding the CC in the autumn. The absence of a soil inversion tillage usually keeps in the topsoil the weed seeds that may germinate the following year (Pekrun et al. 2002). The results from the first year showed that the field on which the innovative technique was applied had a lower weed density than the other fields. In 2024, the technique was applied in the same way as in the first year (field 6_I), which is after rye cultivation and ploughing, but it was also applied on a field that had been cultivated with rice the previous year (field 3_I), followed by a minimum tillage before seeding the winter CC. In field 3_I in 2024, weed density was higher compared to field 6_I, suggesting that the technique itself is not capable of significantly reducing the infestation, as no significant differences were observed in the control areas. Thus, the green mulching technique is probably more effective when integrated with other agronomic practices, but further studies are needed to better understand their role. Ploughing seems to play a core role in reducing weed emergence (Gruber and Claupein 2009), ensuring the effectiveness of the technique. Indeed, the lack of ploughing and a reduced CC development were found to be crucial by organic rice farmers to avoid yield losses (Orlando et al. 2020). However, since agriculture is shifting to more regenerative systems (Kassam et al. 2019), crop rotation is also a key aspect on which farmers can rely to enhance the efficacy of the green mulching technique (Cardina et al. 2002; Koocheki et al. 2009). In particular, rye straw is known to have a suppressive effect on weed emergence when incorporated in the soil (Sung et al. 2010).

The control areas in the field allowed to distinguish the effect of the flooded CC mulching on weed emergence and the effect of flooding water alone. In the first year, significant differences were highlighted in both the traditional and the innovative technique, with a higher density in the control areas. This suggests that weed control is mainly due to the presence of a mulching layer, while the potential phytotoxic effect related to the organic acids released in anaerobic conditions appear to be a less significant contributing factor to weed control, as suggested by Papandrea et al. (2025).

This study allowed to identify which organic acids are mainly released during CC degradation under flooding conditions and their dynamics in the first 10–15 days of flooding. The peaks

usually occur within the first week, but they may vary according to the organic acid considered, to the CC biomass and to the environmental conditions (Rao and Mikkelsen 1977). Acetic and propionic acids were among the most abundant organic acids detected, but a clear behaviour in the release of these two compounds was not observed. In particular, fields with a similar amount of cover crop biomass showed different organic acid release. For example, in 2023, field 3_I showed a higher acetic and propionic acid content compared to field 2_T, despite the similar cover crop development, but the two field had different crop precession (rice in 2_T and rye in 3_I). The same behaviour is observed the following year between field 3_I and 6_I, with the latter showing the highest concentrations, suggesting that rye as the preceding crop could play a key role in organic acid release. The highest concentrations of these acids in the first days of flooding were detected in 2024 (233.9 mg L⁻¹ for acetic acid and 167.4 mg L⁻¹ for propionic acid). Himanen et al. (2012) observed that the germination of cress (*Lepidium sativum*) and ryegrass (*Lolium multiflorum*) was reduced by 50% at acetic acid concentration between about 250 and 380 mg L⁻¹ and at propionic acid concentration between 130 and 280 mg L⁻¹, suggesting that some inhibitory effect on seedlings could be observed at these rates. However, in field condition both rice and weeds are not exposed to a constant organic acid concentration, and weed species may have a different sensitivity, thus further studies are required to focus on the actual effect on weeds.

Organic acids such as acetic, propionic and butyric acids are known to inhibit rice growth, especially roots (Neves et al. 2010; Tunes et al. 2013; Masserano et al. 2022). However, our study compared rice growth only in terms of presence or absence of the mulching layer. In general, little differences were observed in rice growth. Rice height was found to be slightly lower in some control areas, but the reduction of the crop growth is probably linked to the higher infestation in these areas rather than on the presence of organic acids. In both years, a lower SPAD index was observed at tillering in the control areas of the fields on which the traditional technique was applied, highlighting an early weed-crop competition. In the fields managed with the innovative technique, SPAD index was reduced at stem elongation only in 2024; despite the lower weed density in this fields, it is likely that at this stage weeds were more developed and competitive towards the crop. Overall, rice yields were consistent with findings from other studies on organic rice in the same geographical area (Bacenetti et al. 2016; Orlando et al. 2020), but also with the study by Fogliatto et al. (2021), in which the green mulching technique was applied. In our study, the crop yield in the control areas was lower only in the field 1_T in the first year. Since the rice varieties were different between the two farms, yields were not compared between the fields. However, in Farm 2 the same rice variety was employed within the same year. In 2023, the innovative technique field yield was double compared to the traditional technique, confirming a lower weed pressure. In 2024, field 5_T was not harvested, therefore a comparison between the two techniques was not possible, but rice height was overall lower in field 3_I compared to field 6_I, probably because of a higher weed pressure in field 3_I. On the other hand, despite this difference in weed pressure, both the innovative technique fields had a similar yield.

It should be noted that, in the fields where the CC biomass was high, no rice emergence was observed in some areas, especially in the innovative technique (data not shown). A similar harmful effect was observed in the study conducted by Moretti et al. (2025), in which in certain instances the thick mulching layer reduced the number of rice plants by more than 90%.

5 | Conclusions

The green mulching technique is a promising agroecological solution for weed control in organic rice, particularly when integrated with agronomic practices such as ploughing and crop rotation. The CC biomass exerts primarily a physical effect, although flooding leads to the release of organic acids, mainly acetic and propionic acids. Depending on the concentration, these compounds may be harmful to rice and potentially also to weeds, making this aspect worthy of further investigation.

Acknowledgements

We would like to thank the farmers that hosted the field trials: Paolo Maria Mosca, Azienda Agricola di Rovasenda Biandrate, with the support of Stefano Tiraboschi, Riseria Vignola Giovanni and Cerealicola San Giacomo, in particular Sergio Pavese and Andrea Craveris for their precious help and cooperation. We would also like to thank Ivan Di Furia, Amanda Impalea, Marco Signorelli and Cristina Giordano for the collaboration in the field activities.

Funding

The experiment was partially funded by the project “Vantaggi agronomici della pacciamatura verde ed effetto sugli elementi tossici del riso (VERDERISO)” supported by Fondazione CRT (Cassa di Risparmio di Torino). The chemical analyses were carried out within the Agritech National Research Center and received funding from the European Union Next – GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR))—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, CN00000022.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available upon request to the corresponding author.

References

Angeles, O. R., S. E. Johnson, and R. J. Buresh. 2006. “Soil Solution Sampling for Organic Acids in Rice Paddy Soils.” *Soil Science Society of America Journal* 70: 48–56. <https://doi.org/10.2136/sssaj2005.0070>.

Bacenetti, J., A. Fusi, M. Negri, S. Bocchi, and M. Fiala. 2016. “Organic Production Systems: Sustainability Assessment of Rice in Italy.” *Agriculture, Ecosystems & Environment* 225: 33–44. <https://doi.org/10.1016/j.agee.2016.03.046>.

Biswas, J. K., H. Ando, and K. Kakuda. 2001. “Effect of Volatile Fatty Acids on Seedling Growth of Anoxia-Tolerant Rice (*Oryza sativa* L.) Genotypes.” *Soil Science and Plant Nutrition* 47: 87–100. <https://doi.org/10.1080/00380768.2001.10408371>.

Blackshaw, R. E., F. J. Larney, C. W. Lindwall, P. R. Watson, and D. A. Derksen. 2001. “Tillage Intensity and Crop Rotation Affect Weed Community Dynamics in a Winter Wheat Cropping System.” *Canadian Journal of Plant Science* 81: 805–813. <https://doi.org/10.4141/P01-023>.

Bohnen, H., L. S. D. Silva, V. R. M. Macedo, and E. Marcolin. 2005. “Ácidos orgânicos na solução de um gleissolo sob diferentes sistemas de cultivo com arroz irrigado.” *Revista Brasileira de Ciência do Solo* 29: 475–480. <https://doi.org/10.1590/S0100-06832005000300018>.

Camargo, F. A. D. O., E. Zonta, G. D. A. Santos, and R. O. P. Rossiello. 2001. “Aspectos fisiológicos e caracterização da toxidez de ácidos orgânicos voláteis em plantas.” *Ciencia Rural* 31: 523–529. <https://doi.org/10.1590/S0103-84782001000300029>.

Cardina, J., C. P. Herms, and D. J. Doohan. 2002. “Crop Rotation and Tillage System Effects on Weed Seedbanks.” *Weed Science* 50: 448–460. [https://doi.org/10.1614/0043-1745\(2002\)050%255B0448:CRATSE%255D2.0.CO;2](https://doi.org/10.1614/0043-1745(2002)050%255B0448:CRATSE%255D2.0.CO;2).

Chandrasekaran, S., and T. Yoshida. 1973. “Effect of Organic Acid Transformations in Submerged Soils on Growth of the Rice Plant.” *Soil Science and Plant Nutrition* 19: 39–45. <https://doi.org/10.1080/00380768.1973.10432517>.

Council Regulation (EC) No. 834/2007. 2007. “Council Regulation (EC) No. 834/2007 on Organic Production and Labelling of Organic Products and Repealing Regulation (EEC) No 2092/91, No. 834/2007.”

European Union. 2020. “Farm to Fork Strategy. For a Fair, Healthy and Environmentally-Friendly Food System.”

Ferrero, A., S. Fogliatto, A. Barberi, and F. Vidotto. 2021. “Relationship Between Weedy Rice (*Oryza sativa*) Infestation Level and Agronomic Practices in Italian Rice Farms.” *Weed Science* 69, no. 5: 565–574. <https://doi.org/10.1017/wsc.2020.85>.

Ferrero, A., and F. Vidotto. 2007. “Weeds and Weed Management in Italian Rice Fields.” In *Agroeconomical Traits of Rice Cultivation in Europe and India*, 286. Edizioni Mercurio Srl. Research Book. Europe-India Economic Cross Cultural Programme “Rice Net” (EU-India Rice Districts Network Promotion through Agro-Economical, Cross Cultural, and Technical Actions).

Fogliatto, S., L. Patrucco, F. De Palo, B. Moretti, M. Milan, and F. Vidotto. 2021. “Cover Crops as Green Mulching for Weed Management in Rice.” *Italian Journal of Agronomy* 16: 1850. <https://doi.org/10.4081/ija.2021.1850>.

Gruber, S., and W. Claupein. 2009. “Effect of Tillage Intensity on Weed Infestation in Organic Farming.” *Soil and Tillage Research* 105: 104–111. <https://doi.org/10.1016/j.still.2009.06.001>.

Hazra, K. K., D. K. Swain, A. Bohra, S. S. Singh, N. Kumar, and C. P. Nath. 2018. “Organic Rice: Potential Production Strategies, Challenges and Prospects.” *Organic Agriculture* 8: 39–56. <https://doi.org/10.1007/s13165-016-0172-4>.

Himanen, M., P. Prochazka, K. Hänninen, and A. Oikari. 2012. “Phytotoxicity of Low-Weight Carboxylic Acids.” *Chemosphere* 88: 426–431. <https://doi.org/10.1016/j.chemosphere.2012.02.058>.

Kassam, A., T. Friedrich, and R. Derpsch. 2019. “Global Spread of Conservation Agriculture.” *International Journal of Environmental Studies* 76: 29–51. <https://doi.org/10.1080/00207233.2018.1494927>.

Khan, Q. A., and K. A. McVay. 2019. “Productivity and Stability of Multi-Species Cover Crop Mixtures in the Northern Great Plains.” *Agronomy Journal* 111: 1817–1827. <https://doi.org/10.2134/agronj2018.03.0173>.

Koocheki, A., M. Nassiri, L. Alimoradi, and R. Ghorbani. 2009. “Effect of Cropping Systems and Crop Rotations on Weeds.” *Agronomy for Sustainable Development* 29: 401–408. <https://doi.org/10.1051/agro/2008061>.

- Le Mer, J., and P. Roger. 2001. "Production, Oxidation, Emission and Consumption of Methane by Soils: A Review." *European Journal of Soil Biology* 37: 25–50. [https://doi.org/10.1016/S1164-5563\(01\)01067-6](https://doi.org/10.1016/S1164-5563(01)01067-6).
- MacLaren, C., P. Swanepoel, J. Bennett, J. Wright, and K. Dehnen-Schmutz. 2019. "Cover Crop Biomass Production Is More Important Than Diversity for Weed Suppression." *Crop Science* 59: 733–748. <https://doi.org/10.2135/cropsci2018.05.0329>.
- Masserano, G., B. Moretti, C. Bertora, et al. 2022. "Acetic Acid Disturbs Rice Germination and Post-Germination Under Controlled Conditions Mimicking Green Mulching in Flooded Paddy." *Italian Journal of Agronomy* 17: 1926. <https://doi.org/10.4081/ija.2022.1926>.
- Meryemoglu, B., B. Nis, and B. K. Ozsel. 2023. "Organic Acids Production From Low-Cost Cellulosic Substrates by Fermentation." *Biomass Conversion and Biorefinery* 14: 17019–17028. <https://doi.org/10.1007/s13399-023-03826-5>.
- Moretti, B., F. Vidotto, C. Grignani, D. Said-Pullicino, and S. Fogliatto. 2025. "Cover Crops, Soil Fertility and Straw Management Influence Rice Nutrition, Weed Competition and Greenhouse Gas Emissions in a Mesocosm Study." *Italian Journal of Agronomy* 20: 100037. <https://doi.org/10.1016/j.ijagro.2025.100037>.
- Neves, L. A. S., C. Bastos, E. P. L. Goulart, and C. E. F. Hoffman. 2010. "Physiological Quality of Seeds of Lowland Rice Submitted to Organic Acids." *Revista Ciencia Agrovet* 9: 169–177. <https://revistas.udesc.br/index.php/agroveterinaria/article/view/5297>.
- Oliveira de Sousa, R., M. do Carmo Ruaro Peralba, and E. J. Meurer. 2002. "Short Chain Organic Acid Dynamics in Solution of Flooded Soil Treated With Ryegrass Residues." *Communications in Soil Science and Plant Analysis* 33: 779–787. <https://doi.org/10.1081/CSS-120003065>.
- Orlando, F., S. Alali, V. Vaglia, et al. 2020. "Participatory Approach for Developing Knowledge on Organic Rice Farming: Management Strategies and Productive Performance." *Agricultural Systems* 178: 102739. <https://doi.org/10.1016/j.agsy.2019.102739>.
- Papandrea, G., S. Fogliatto, F. D. Palo, and F. Vidotto. 2025. "Green Mulching of Cover Crops in Rice: Effects on Weed Germination and Emergence." *Advances in Weed Science* 43: e020250037. <https://doi.org/10.51694/AdvWeedSci/2025;43:00037>.
- Pekrun, C., A. El Titi, and W. Claupein. 2002. "Implications of Soil Tillage for Crop and Weed." In *Soil Tillage in Agroecosystems*. CRC Press. <https://doi.org/10.1201/9781420040609>.
- Pinheiro, J., and D. Bates. 2025. "nlme: Linear and Nonlinear Mixed Effects Models R Package Version 3.1-164."
- R Core Team. 2025. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Rao, D. N., and D. S. Mikkelsen. 1977. "Effects of Acetic, Propionic, and Butyric Acids on Rice Seedling Growth and Nutrition." *Plant and Soil* 47: 323–334. <https://doi.org/10.1007/BF00011491>.
- Sung, J.-K., J.-A. Jung, B.-M. Lee, et al. 2010. "Effect of Incorporation of Hairy Vetch and Rye Grown as Cover Crops on Weed Suppression Related With Phenolics and Nitrogen Contents of Soil." *Plant Production Science* 13: 80–84. <https://doi.org/10.1626/pps.13.80>.
- Tsutsuki, K., and F. N. Ponnampereuma. 1987. "Behavior of Anaerobic Decomposition Products in Submerged Soils: Effects of Organic Material Amendment, Soil Properties, and Temperature." *Soil Science and Plant Nutrition* 33, no. 1: 13–33. <https://doi.org/10.1080/00380768.1987.10557549>.
- Tunes, L. M. D., L. C. Tavares, D. Â. R. Fonseca, A. C. S. A. Barros, and C. D. A. Rufino. 2013. "Effect of Organic Acids in Physiological Quality of Rice." *Ciência Rural* 43: 1182–1888. <https://doi.org/10.1590/S0103-84782008005000029>.
- Uno, T., R. Tajima, K. Suzuki, M. Nishida, T. Ito, and M. Saito. 2021. "Rice Yields and the Effect of Weed Management in an Organic Production System With Winter Flooding." *Plant Production Science* 24: 405–417. <https://doi.org/10.1080/1343943X.2020.1865823>.
- Vaglia, V., J. Bacenetti, F. Orlando, S. Alali, E. Bosso, and S. Bocchi. 2022. "The Environmental Impacts of Different Organic Rice Management in Italy Considering Different Productive Scenarios." *Science of the Total Environment* 853: 158365. <https://doi.org/10.1016/j.scitotenv.2022.158365>.
- Vitalini, S., F. Orlando, V. Vaglia, S. Bocchi, and M. Iriti. 2020. "Potential Role of *Lolium multiflorum* Lam. in the Management of Rice Weeds." *Plants* 9: 324. <https://doi.org/10.3390/plants9030324>.