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NEW AGROTECHNICAL RESEARCH FOR ORGANIC RICE PRODUCTION AND THEIR SUSTAINABILITY ASSESSMENT

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Sommario

Introduction

This thesis studies and evaluates the agronomical techniques associated with organic rice cultivation in the Northern Italy area. The study funding is from the Italian MIPAAF project "Risobiosystems", which started in 2017 and ended in 2020.

The studies presented are connected to the European sustainable food production strategy "Farm to Fork," which is the core of the European Green Deal to make food systems fair, healthy and environmentally friendly, encouraging the expansion of the organic agriculture sector (European Commission 2020). An increased transfer of knowledge from organic to conventional agriculture and vice versa could be possible in the future.

As reported in the literature, organic farming is capable of reducing the environmental impact of agriculture by avoiding the use of synthetic compounds (e.g. fertilisers, pesticides) and by promoting practices (e.g. crop rotation, leguminous cultivation, organic fertilisers, green manure crops, green mulching.) able to increase the soil carbon stock, and prevent the indirect environmental impacts due to the industrial production of inputs (Acuna et al. 2018). Organic agriculture produces biodiversity, with increases of abundance and species richness observed for birds, mammals, invertebrates and flora (Hole et al., 2005), shows higher economic values concerning some ecosystem services (Sandhu et al. 2008), and leads to a decrease of nitrate concentration into the water (Honisch et al., 2002). For rice, in particular, the organic system was observed able to increase the soil carbon storage capacity (Komatsuzaki and Syuaib, 2010) and organic matter content, facilitating the soil preparation (Mendoza, 2004), and promoting the ecological succession and temporal heterogeneity of the macrophyte communities into the soil (Martínez- Eixarch et al.; 2017).

The thesis is a collection of articles published or under review associated with the organic rice production connected with Risobiosystems project results. Each paper explains my work and contribution, and each Chapter gave rise to new and ongoing studies.

The presented papers are the starting point for further research to continue providing evidence and scientific results for the future of organic farming.

Chapter I reveals the lack of scientific research and information about organic rice farming, especially in Europe and Italy. This thesis is providing a new step forward for the sector.

Therefore, each Chapter includes an article or a manuscript that testifies the diversity of working groups I have collaborated with and the different study tools addressed. The introduction to each Chapter facilitates the connection between the works and indicates the in-depth studies carried out that are still under development. Figure 1 will shortly represent the connection and the main topic presented in this thesis.

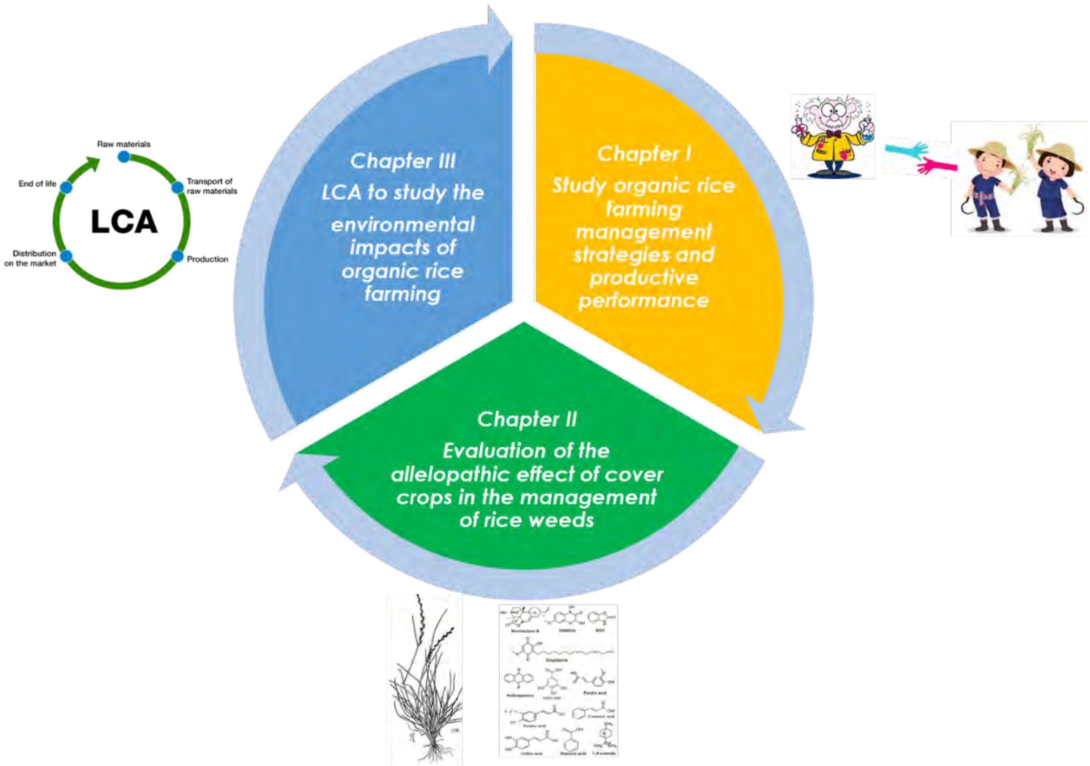

Figure 1 A graphical representation of the thesis chapters

The studies mainly propose three different weed management techniques in the paddy field that can be considered models that can be standardised on farms specialising in their use. These models (deepen in Chapter I) are the basis of a sustainable fight against ecological and environmental problems thanks to the strict avoidance of chemicals such as herbicides. Value is given to methodologies whose effectiveness has been widely tested, first of all, the false sowing technique in dry and in water condition: working the soil in advance, it favours the birth of weeds, which are then removed through agricultural operations and subsequently the rice is sown in soil whose seed bank has shrunk. On farms where there is less water available in the area, it is also possible to remove the weeds using the harrow as long as the phenological phases of the rice allow it. Another proposed technique is green mulching, an innovative method based on an ancient technique to control weeds recently introduced in numerous variants in Italian rice farms but whose use has not been extensively studied.

The green mulching technique involves using cover crops that are effective in controlling weeds thanks to four main mechanisms. The first consists of the partial inhibition of weeds' germination thanks to competition for water, nutrients, shading, which are phenomena caused by the presence of cover crops. The second consists of the mulching effect when the crops are chopped or placed on the ground in conjunction with rice sowing. The third is represented by the phenomena of allelopathy that arise between cover crops and weeds (this aspect will be further explored in Chapter II). At the same time, the fourth mechanism is linked to the accumulation of phytotoxic compounds following the fermentation caused by the submersion water of the paddy field: the anaerobic environment that is created leads to the formation of organic acids such as acetic acid, butyric and propionic acid. The studies also presented from the output of Risobiosystems proposes different management scenarios for green mulch according to the territorial and environmental characteristics. First of all, there are variants regarding the types of cover crops used: sowings can be carried out in purity using rye, triticale, soft wheat, rapeseed, clover, vetch and above all, ryegrass, or they can be carried out in a mixture using ryegrass together with triticale, rapeseed and vetch or ryegrass together with clover and vetch in various proportions.

Regarding the sowing time, it is important to ensure good emergence and biomass growth of the cover crops, and in the case of ryegrass, for example, it is advisable to proceed with sowing by the end of September. There are various combinations for the sowing and subsequent treatment of the grass crops: row sowing followed by shredding, broadcast sowing followed by rolling / shredding, broadcast sowing followed by rolling or broadcast sowing and crops left standing. As far as rice management is concerned, sowing can occur on standing or rolled plants. In the first case, the cover crops are subsequently shredded, rolled, or left standing as previously described. However, there are two main models of green mulch management. The first involves the sowing of broadcast rice between the standing grass shed, then the cover crops are chopped, and the paddy field is submerged. Fermentation thus takes place when the rice is at the germination stage. The second involves the sowing of rice in rows always between the standing grassland followed by a dry period of up to 20-30 days, but there may be short intervals of low submersions as needed. Subsequently, the weed is knocked down and submerged, and in this case, the fermentation occurs when the rice is at the third-fourth leaf stage. Fermentation must be carefully monitored to avoid repercussions on rice, which would otherwise be damaged. A signal that suggests stressful conditions is the white and curled coleoptile, which means that it is time to stop submersion and make a change of water. Even the colour of the water itself highlights the excessive presence of organic acids and bacterial formations: it is advisable to avoid reaching brick red colours. In conclusion, green mulching is a sustainable and innovative technique that combines various mechanisms that effectively suppress - at least in part - weeds.

Allelopathy is an interesting aspect offered by plants in this particular agrotechnical. Allelopathy generally produces and releases secondary metabolites, generating inhibitory effects against nearby plant species. The germination, growth and reproduction of target plants can thus be impaired; these aspects were deepened in Chapter II starting from the organic farmers' experience. The study aimed to define the inhibitory action of *Lolium multiflorum* Lam., used as a cover crop before rice sowing against *Echinochloa oryzoides* (Ard.) Fritsch is one of the main rice weeds. Chapter III focuses on evaluating the environmental impact of organic rice cultivation through LCA considering the production scenarios and the agrotechnics described in Chapter I.

The LCA approach was adopted because it is largely used to assess the environmental impact of the agriculture process. However, from an LCA viewpoint, organic agriculture is not an obvious answer to environmental problems because LCA defines the function of the studied system using a 'functional unit', which should be a precise measure of what the system delivers but is not able to consider for example indirect effects. Furthermore, LCAs express impacts per unit of a product by default. However, organic agriculture generally emits fewer pollutants per unit of land occupied than conventional agriculture (an area-based approach); it may have higher impacts per unit of product due to its lower yields per unit area (van der Werf, 2020).

Chapter I

In Organic Agriculture, participatory research finds a leading role as it is essential to draw up and be able to continuously update standardisable manuals to summarise good agronomic practices to be applied. In the case of organic farming, there is currently not enough theoretical and practical knowledge of standard farming methods for conventional farming. The greatest knowledge on practices and techniques is relegated to farmers who risk experimenting on themselves with new ideas. Collaborative research with farmers can help promote the most useful agro-techniques and methods mainly used for farming based on context.

Especially in Italy, it is easier for farmers to collaborate with research institutions through a bottom-up approach that allows for a better connection between the figures avoiding hierarchical structures and having a peer to peer connections (Barbercheck M. et al. 2014).

During the beginning of the PhD, I collect data with interviews, surveys of the weeds in rice fields, and yield data collection at the harvest.

My work was part of a process started two years earlier and allowed the group to conclude with the scientific work Orlando et al. 2019, which is the first testimony in Italy of a participatory research experience in organic rice production. The main objectives of the study are focused on:

- knowing which are the most used techniques in Northern Italy for rice cultivation according to the organic method;
- measuring the productive scenarios of organic rice in North Italy;
- understanding the strengths and weaknesses of the different techniques applicate by farmers;

The network of professionals, farmers and researchers involved in this study is extensive. Collaborating with a researcher from different disciplines made it possible to publish a further work I contributed. That work concerned the description of the components of the research network by investigating the emotive and psychological aspects of participatory research towards farmers (Pagliarino, E., Orlando, F., Vaglia, V. et al. Participatory research for sustainable agriculture: the case of the Italian agroecological rice network. Eur J Futures Res 8, 7 (2020). Https://doi.org/ 10.1186 / s40309-020-00166-9). This last work was an inspiration to co-supervise a three-year internship, which is in progress, to elaborate and administer a questionnaire addressed to the farmers of the network to capture the strengths and weaknesses of participatory research in agrarian context to improve its efficiency and provide critical and strong points to the institutions that currently encourage the participatory research methodology in research calls.

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Participatory approach for developing knowledge on organic rice farming: Management strategies and productive performance

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ABSTRACT

Rice is the third grown crop worldwide and responsible of significant environmental impacts. Nevertheless, there is a lack of knowledge concerning the organic rice' performance and management, probably due to the limits encountered by the reductionist approach in studying complex systems such as an organic paddy. The study proposes a knowledge-intensive and qualitative research methodology based on researcher-farmer participatory approach, with the aim to improve the state of knowledge on organic rice, explore the yield potential and variability, and identify the successful agronomic practices. A wide range of cropping systems placed in North Italy were monitored and analysed during three years by a multi-actor network. Knowledge was generated from collected data and information, integrating the scientific and empirical knowledge on the basis of the DIKW hierarchy and through mutual learning and knowledge sharing tools. The organic rice field proved to be a complex and difficult to predict system, which evolves over the time, under the on-going pressure of the bottom-up innovations, and whose performance depends on many interacting elements. The results highlighted three main knowledge-intensive management strategies, not involving universal recipes but a range of agroecological principles and flexible solutions that the farmers adapt to the time- and space- variability through an active adaptive management. Yield showed a wide variability (0–7 t/ha) and normal distribution (median 4 t/ha). The lower, middle and upper quartiles of yield showed a mean of about 2, 4 and 6 t/ha, respectively, with high variance associated with upper and lower quartiles. The variability sources related to the management and effectiveness in weed control have mainly determined the productivity gap, "Know-how" (suitability of the chosen management plan), "optimization" (timely and accuracy of interventions) and "seed bank" (previous operations and land uses affecting the weeds dynamics) were responsible of the low yield in 77%, 54% and 31% of the cases, respectively, drowning out the impact of climate, soil and variety.

Results are useful to drive further scientific inquiries and evaluations consistently with the faced reality by the farmers, and suggest that improvements in the farmer' know-how and skills can lead to further yield increase and variability reduction. The participatory research, adopted to explore complex systems, has worked in this direction, fostering the co-creation of knowledge and innovation and the social cohesion. However, the methodology showed constraints mainly related to the time-consuming surveys and its nature affected by human component.

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1. Introduction

1.1. State of knowledge on organic rice

Italy is the first rice producer in Europe, accounting for the 52% of the European rice area (FAO, 2016), with 234,000 ha. The latter are mainly based on high-input monoculture and concentrated in one of the most profit-yielding rural area of Italy, between Lombardy and Piedmont regions. In the recent years, the national rice sector was involved in environmental and socio-economic issues. Public authorities (Ispra, 2018) reported the higher degradation of water quality in rice production areas, due to contamination by pesticides, with concerns for the integrity of the environment and public health. In the same time, a significant price decrease occurred for conventional rice due to the competition with imported quantities (e.g. in 2017, Copa Cogeca, the European Association of Farmers and Agri-Cooperatives, asked for the declaration of state of crisis and special subsidies for rice farmers). In this context, organic agriculture can be a solution, in favour of both environmental and economic sustainability, avoiding the use of herbicides, and then reducing the impact on water quality, and providing the farmers an alternative based on higher price paid by the market for organic products.

However, organic agriculture is a novelty and a challenge for this sector. Farmers, who are familiar with industrialized and high-input agriculture, perceive the conversion to organic as difficult to implement, risky and uncertain in terms of productive performance. They are reticent to significantly change the management practices, operation schedules and farm structure (e.g. introduction of crop rotation or cover crop, purchase of new machines for weed control). The lack of skills, necessary to control weeds without the use of herbicides, and the uncertainty, concerning the organic rice cropping system' (ORCS) performance and behaviour, are the main constraints. All this is accompanied with the absence of systematic knowledge and external supporting expertise as technicians or researchers.

In fact, weed control remains the major challenge for organic systems (Shennan et al., 2017). Rice is particularly prone to weed issues and the intense weed competition is the main constrain to realize the potential production (Hazra et al., 2018), in addition, the weed incidence is reported as the main causes of yield variability and yield gap with conventional rice in Mediterranean organic paddies (Delmotte et al., 2011).

Nevertheless, few scientific publications have addressed this topic, resulting in a lack of knowledge about the strategies for weed control and the resulting yield. Most of the scarce literature on organic rice is focused on the dynamics of nutrients with organic manures, the greenhouse gas emissions balance and the genotype (Hazra et al., 2018). While, systematic researches on the practices, productive inputs and performance of the ORCSs are lacking (Huang et al., 2016). Only 17 recent papers (since 2004) were found by SCOPUS entering the key words “organic rice”, “yield”, “weed”, while 1,389 papers (since 1952) entering the key words “rice”, “yield”, “weed”. The missed understanding of the management strategies for the ORCS, and the corresponding yield potential and variability, is a constraint in encouraging the conversion of conventional farmers. This condition makes the organic rice cultivation a niche activity, carried out by few pioneer farmers with a self-help approach. As shown by the literature, the diffusion of organic farming in a new productive sector or countries is a slow process, mainly driven by the farmers themselves (Padel, 2001; Kroma, 2006; Ortolani et al., 2017). Farmers are the first innovators and experimenters, developing on-farm new techniques and skills, with a trial-and-error approach. Therefore, in organic agriculture, the innovation development often follows the “bottom-up” paradigm, instead of a “top down” linear process.

1.2. Research approach for complex systems

Researchers could have a pivotal role in speeding up the transition process toward organic agriculture, supporting the dynamics of “bottom-up” innovation, but a profound change is required in the practice of research, since the organic farming and its management differ a lot than the conventional farming. The industrial agriculture tries to do the same in different places and in the same places (e.g. monoculture, universal agronomic recipes), involving simplified and specialized cropping systems managed with a short-term approach and based on the fast action of external inputs. On the other hand, organic agriculture involves complex systems, regulated by long-term biological processes and cumulative and non-linear effects (e.g. humus formation, weed seed bank dynamics), where the effectiveness of an agricultural practice is time- and site- specific and greatly dependent on the historical causalities of each production situation (Duru et al., 2015).

Facing the above mentioned uncertainty, an active and adaptive management may underlie the decision making process of the organic farmers, with a deliberate plan for learning about the managed system (Shea et al., 2002). The adaptive management is pointed out by the agroecological studies, as the farmer' response to across time- and space- variability (Bell et al., 2008), based on common ecological and agronomic principles that underlie a variety of practices, rather than on a unique and standard management and context-independent recipes. Therefore, the farmer' decision making process leads to agricultural practices highly variable in time and in space, depending on the site-specific features of the farm and the cultivation environment, and on the contextual occurrences during the growing season. The ORCS management is complex, as complex is the addressed system, and in accordance with the agroecological vision (Bell and Bellon, 2018), the pioneers of this sector are trying to do different things in different places, and different things in the same place (e.g. crop rotation, wide range of agronomic solutions and variants of the same practices). The adaptive management is farmer-centred and involves will-known complex practices, which can be acquired through direct experience, or mutual-learning and sharing experiences within other farmers. However, this knowledge is difficult to validate within the prevailing institutional norms of research that accords value to objective and standardized knowledge (Kroma, 2006).

In this context, the research, traditionally targeted to solve the industrial agriculture issues, is based on a reductionist approach that is uncomfortable in studying complex systems characterized by many interactions and variability sources (Drinkwater, 2002; Ponzio et al., 2013; Duru et al., 2015; Döring, 2017). The factorial experiments aim to deconstruct the complexity of the system, reducing the variability and isolating the causal relationships among few elements. This approach could be unable to predict the performance of the whole agroecosystem, leading to incorrect conclusions or results that are not replicable or confirmed by the reality on the ground (Drinkwater, 2002; Baker, 2016; Carberry, 2001; Sadras and Denison, 2016; Hazra et al., 2018). As shown by Kravchenko et al. (2017), in conventional farming, the recorded yields in experimental plots are often in line with those obtained in the farm' fields under the same management treatment, while in the organic farming a persistent yield gap can be observed between them. The challenges that associated with weed control and the timeliness of the management interventions were found to be the cause of the lower yield of the organic fields. Similarly, Stoop et al. (2009) highlighted the weakness of the results coming from the rice trials performed by IRRI (International Rice Research Institute), targeted to find standardised solutions suitable for a linear technology transfer process among farmers. This approach neglects the variability and diversity encountered in the real-world farming environment, excluding the interactions between experimental and non-experimental factors. The latter factors, kept constant during the experiments, play instead a critical role in determining the rice yield. The authors highlight the limits of the current research, asking to scientific community for more flexible solutions originated from knowledge-intensive agronomic practices.

In order to produce suitable agronomic solutions and comprehensive results for organic farming, the research in agriculture need to be reconsidered, moving beyond the plot-scale, toward a holistic vision, on-farm studies, the action research (Bengtsson et al., 2005; Shennan et al., 2017; De Ponti et al., 2012), and the use of interdisciplinary tools and participatory approaches

(Rockström et al., 2009; Drinkwater, 2002; Ponzio et al., 2013). In particular, higher level of farmers' participation into the research, and the integration of farmer's expert knowledge into a bottom-up innovation process are required (Carolan, 2006; Ingram, 2008).

However, agricultural research is still far from integrating the traditional tools with a holistic and interdisciplinary vision, and unable to cooperate with farmers in a process of peer-to-peer knowledge exchange and mutual learning (Kroma, 2006; Méndez et al., 2015). On the other hand, agroecology has well-recognized this need, and thus proposes the participatory networks of farmers and scientists as main tool for understanding the complexity of agroecosystems and food systems (Warner, 2008; Méndez et al., 2013, 2015; Berthet et al., 2016). Many studies showed as the participatory approach and the incorporation of local knowledge into the process of scientific inquiry, led to a more complete understanding of natural and agricultural systems, in favour of their sustainable management, starting from the assumption that people can have well-founded understanding of their own environment. Especially in wetlands, where water adds an upper level of complexity, the research' need to recur to helpful perceptions from the local community, seems to be exacerbated (Calheiros et al., 2000; Ramsar, 2000; Barzman and Desilles, 2013; Stoop et al., 2009). In according with the five levels of change toward agroecology (Gliessman, 2016), the participatory research proved to be able to foster the transition beyond the field and farm level. As shown by successful researches in plant breeding (Murphy et al., 2005; Mancini et al., 2017; Ortolani et al., 2017), food network building (Guzmán et al., 2013) and rural development (e.g. FAO projects), the participatory approach supports the sustainable development of local and global food systems, social innovation, and farmers' empowerment. Lilja and Bellon (2013) highlight how participatory studies are needed when the agriculture development requires a holistic vision (i.e. change of the whole cropping system, rather than one technology at a time), and the crop growing conditions vary widely among farmers and sites. This approach was often tried after the failure of the traditional scientist-designed research programmes in the complex and risk-prone contexts of the poor and marginal agricultures, characterized by high variability and the need to improve the natural-resource management. Today, the participatory research can be a valuable tool also for the organic agriculture of the industrialized countries that has many features in common with the low-input farming systems of the developing countries.

However, the methods of participatory research are not commonly employed to develop innovation in agriculture and the scientists remain sceptical of formally incorporating the knowledge of local people. As result, a substantial research gap exists in the understanding of complex farming systems, such as those of flooded organic rice fields, and in aligning of modern agricultural techniques with them, in order to support solutions useful for organic farmers (Hazra et al., 2018).

1.3. Precondition of the participatory research

The present study addresses the rice' cultivation area of North Italy. This case study provided an encouraging context for a farmer-researcher participatory exploration of complex systems. The researchers' intention to improve the current state of knowledge on the ORCS (i.e. input, management and performance), in order to perform further evaluations (e.g. environmental, economic and agronomic) coherent with the real-world farming environment, met the needs of the local organic farmers. The latter demonstrated the will to improve their management, through the experiences and skills sharing, and to contrast the widespread scepticism among the scientific and rural community, which can be represented by the following sentences "Producing rice without herbicides is impossible", "Organic farming is unable of satisfactory economic returns", "Organic farming increase the environmental polluting, because the fuel consumption for mechanical weeding and the lower yield". These farmers represent for scientists a precious source of knowledge, generated by the active adaptive management, through the learning of suitable practices by trial and error over the years and the implementation of locally relevant empirical knowledge.

1.4. Study goals

Starting from the needs of scientists and farmers, the present study addresses the challenge of integrating the farmers' insights, perceptions and knowledge into the process of research, exploring the complex agroecosystem of organic rice field. At this aim, a methodological framework, based on the Data-Information-Knowledge-Wisdom (DIKW) hierarchy (Ackoff, 1989) and participatory tools, is proposed. For three years, ten farmers were actively involved in the research on ORCS, and in a multi-actor network (namely: the organic rice network; OR-Net) composed of other farmers, scientists and sector' technicians. Farmer-led experiments were monitored and evaluated by the OR-Net, taking into account real agro-ecosystems, with different features in terms of farm size, crop management, and constraints faced by the farmer.

The main study goal was to improve the state of knowledge on the ORCS, providing results useful to develop, without approximations and contextually, any further evaluations (e.g. economic, productive or environmental), and to make the transition to organic agriculture less uncertain. The following sub-goals were addressed:

i) Define and evaluate the most promising management strategies (identifying the agricultural practices, input, functional principles, and performing a participatory SWOT analysis).
ii) Explore the productive performance of a broad range of ORCSs (analysing the yield range and the main sources of the variability).
iii) Make considerations (opportunities and constraints) on the participatory approach for the explorative study of complex agro-ecosystems.

2. Materials and methods

2.1. Organic rice network: farmers and farms features

During the growing seasons (i.e. 2016, 2017, 2018), ten farmers, that grow organic rice in North Italy, were involved into the participatory research on ORCSs. The related farms are placed between the provinces of Vercelli (Piedmont region) and Pavia (Lombardy region), where the main Italian rice production is concentrated (i.e. 67% of the national harvests; Istat, 2016). Farmers were identified on the basis of the information framework on the local reality of organic sector, coming from: i) agronomists and technicians with a deep knowledge of the territory, because in charge of further farm' inspections, beyond those carried out by the certification body (involved in plans to limit fraud in organic sector, by private companies and regional authorities); ii) local pioneers of the organic rice cultivation, with a pre-existing relationship of trust with the research team (i.e. ex-student); iii) the farmers, progressively included into the OR-Net, since nobody like the organic farmers know which farmers embrace the organic agriculture, beyond the economic reasons, to support a system of values (i.e. environment protection and new models of food production). Then, farmers available to collaborate for a common purpose in favour of

Table 1
Features of monitored farms. Legend: P = Pavia Province (Lombardy); V = Vercelli Province (Piedmont); M = male; F = female; UAA = Utilised Agricultural Area; Y = yes.

Farm ID	Site	Gender	Testing organic since	UAA (ha)	Organic crops (% UAA)	Rice (% UAA)	Set-aside	Rotation	Rotational Crops		
									Legumes	Cereals	Other crops
1	P	M	1976	476	100	29	Y	Y	soybean, pea	maize, rye	rapeseed
2	P	F	2006	106	100	30	Y	Y	soybean, bean, field bean	barley, spelt, triticale, wheat	buckwheat, rapeseed, sunflower
3	P	M	2008	13	100	12	Y	Y	bean, pea	maize	
4	P	F	2008	29	100	24	Y	Y	soybean, pea	mile, spelt	buckwheat
5	P	F	2016	103	100	18	Y	Y	alfalfa	maize	
6	P	M	2016	210	14	40	Y	Y	soybean	maize, barley, rye	
7	V	M	2015	125	100	80	Y				
8	V	F	2015	82	100	46	Y	Y	soybean		
9	V	M	2015	33	100	64	Y	Y	soybean		
10	V	M	2016	64	100	50	Y	Y	soybean		

organic sector were recognized, avoiding possible distortion in the study results due to the inclusion of fraudulent farmers.

Those farmers were involved into the multi-actor network OR-Net, which its core composed of the farmers themselves, three technicians and three researchers in agronomy. During the study, the OR-Net progressively involved scientists from other disciplines (i.e. in agreements with the research' needs of expert knowledge) and other farmers, in order to extend the debate and exchange of opinions on ORCS.

The high level of farmers' participation, needed for generating new knowledge (Lilja and Bellon, 2013), was assured. Peer-to-peer relationships from collaborative to collegial were established between researchers and farmers, both partners in the research process. The informal and local R&D system was encouraged, as well as the knowledge sharing and mutual-learning system, underlying the methodological approach (sections 2.3,2.4).

The main features of the ten farms involved in the study are listed in Table 1. Four out of the ten are managed by women, a significant share, considering that the local agriculture is mainly managed by men (e.g. in Lombardy 78.2% of farm's heads are men; Istat, 2010). This is in accordance with the active role of women, highlighted by Padel (2001), in developing and sharing innovation for organic farming. The farmer's experience level in organic cultivation was different: four farmers (i.e. farm ID 1, 2, 3, 4) with at least 8 years of experience in organic farming, and the remaining at their early stage (i.e. in 2016: three farmers were at the first year, and three farmers at the second year of experience). The farm Utilized Agricultural Area (UAA) ranges between a maximum of 476 ha and a minimum of 13 ha, with half farms having UAA more than 100 ha (i.e. farm ID 1, 2, 5, 6, 7), and the remaining below. Most of farmers are testing organic system in the entire farm, except one (i.e. farm ID 6) who maintained a mixed organic-conventional regime. Most of farmers introduced crop rotation with no more than two-thirds of UAA dedicated to rice, except one (i.e. farm ID 7) who alternates rice growing in monoculture with set-aside land. The four oldest pioneers (i.e. farm ID 1, 2, 3, 4) adopt complex plane of crop rotation, with the introduction of cereal and minor crops in addition to legumes, while most of remaining farmers alternate a year of rice with one year of legumes, mainly soybean.

2.2. Organic rice cropping systems

A heterogeneous sample of 50 ORCSs were monitored (i.e. 15 in 2016, 22 in 2017 and 13 in 2018). The management, planned by the farmers on the basis of their adaptive strategies, has been kept unaltered. Each ORCS was thus evaluated as a whole agroecosystem, using a holistic approach, closely aligned with the study of natural ecosystems. Since the variables of the system and the sources of variability were not isolated, a knowledge-intensive and qualitative research methodology option was used for the assessment (see section 2.3,2.4). The ORCSs' features are shown in Table 2. The fields have been chosen on the basis of their representativeness in terms of management practices adopted by the farmer, and excluding those usually characterized by yield above or below the farm' average for reasons due to soil properties or other external factors.

Direct seeding of rice and the flooded conditions of growth were common elements for all the ORCSs. Three main strategies were used to manage the ORCSs, mainly targeted to weed control (see Table 2, Testing techniques: SD = Stale seedbed in Dry paddy, mainly using comb harrow; SF = Stale seedbed in Flooded paddy, using different types of machines; CC = use of green mulch from different Cover Crops). The strategies were used as unique treatment or in combination. The ORCSs differed also by the used varieties: the japonica genotype "Rosa Marchetti" (Callegarin et al., 1994) was grown by five farmers out of ten, while the "Ronaldo" (Ilieva et al., 2017) was grown by other three. In each rice field, soil sampling (5 sampling points of the upper soil 30 cm) and the related standard physical analysis were carried out. Results confirmed those of other authors (Tanaka et al., 1973), revealing the tendency of rice fields placed in Pavia Province to have a higher percentage of sand (i.e. prevalence of sandy-loam soils), while a decrease of sand percentage and an increase of silt for paddies placed in Vercelli Province (i.e. prevalence of silty soils).

2.3. The methodological approach

The Data-Information-Knowledge-Wisdom (DIKW) hierarchy (Ackoff, 1989) was used to explain the nature and the obtaining pathway from the collected data and information, to the generated results (namely the knowledge) on the ORCS. The DIKW hierarchy, also known as "Knowledge Pyramid", is often used in the field of information systems and knowledge management. For this reason, it is suited to describe the used methodology based on participatory and typically knowledge intensive approach. The conceptual chart of Fig. 1 contextualizes "data", "information" and "knowledge" following the DIKW pyramid (Fig. 1, on the left), and showing processes (Fig. 1, on the right) and tools (Fig. 1, in the middle) involved in the transformation of an entity at a lower level in the hierarchy (e.g. data), into that at a higher level (e.g. information). The main entities of the DIKW hierarchy, and the processes of shift among levels, as reported in the Rowley (2007) and Wiliams (2014) reviews, are described below.

Data mainly deal with description of the parts. Then, data are objective facts, elementary observations and concerning the properties of things, events and activities. Data alone are not able to be useful for a purpose (i.e. data as "Know nothing").

Information mainly deals with the understanding of the relations between parts. The information resides in the mind of the recipient and adds value to the understanding. Information are data that were processed or interpreted, so that data assumes a meaning for a specific

Table 2
Features of monitored organic rice cropping systems. Legend: SA-LO = sandy-loam; SI-LO = silty-loam; LO-SA = loamy-sand; SI = silt; Y = yes; SD = Stale seedbed in Dry paddy, in combination with comb harrow; Stale seedbed in Flooded paddy, in combination with minimum tillage machines; CC = Flooding of green mulch from different Cover Crops.

Farm ID	Rice varieties		Soil texture	Testing techniques			Monitored fields (n.)		
	Name	n°		SD	SF	CC	2016	2017	2018
1	Rosa Marchetti, Ronaldo, Baldo	3	SA-LO	Y	Y	Y	2	2	2
2	Rosa Marchetti, Ronaldo, Loto	3	SI-LO	Y	Y	Y	3	3	3
3	Ronaldo, Loto, Tondo cerere	3	SA-LO	Y			1	1	1
4	Carnaroli, Ermes, Venere	3	SA-LO	Y			2	2	1
5	Sant'Andrea, Baldo	2	SA-LO			Y	0	4	2
6	Sant'Andrea	1	LO-SA			Y	1	1	0
7	Rosa Marchetti, Pato	2	SI		Y	Y	2	3	1
8	Carnaroli	1	SI-LO			Y	3	3	2
9	Rosa Marchetti	1	SI-LO			Y	0	2	1
10	Rosa Marchetti, Carnise	2	SI-LO		Y	Y	1	1	0

purpose, in order to be useful, providing answers to "who" "what" "where" and "when" questions (i.e. information as "Know what").

Knowledge mainly deals with the understanding of the patterns (i.e. parts as a whole). Knowledge is a property of people, generated from the integration of contextual data and information with expert opinion, skills, training, own or other experience, perception, common sense and the background knowledge. Therefore, knowledge involves the prior understanding and accumulated learning, and the reflection and synthesis of information from multiple sources over time. During the knowledge generation' process new insights are internalized by establishing links with already existing knowledge; these links can range from firmly characterized relationships to vague associations. Finally, knowledge involves as a potential for action "the know-how", which is originated from the transformation of data and information into guidelines, and then results in a valuable asset useful for decision making and to increase the capacity to take effective action (i.e. knowledge as "actionable information" or "information combined with understanding and capability"). Knowledge provides answers to "how" questions (i.e. knowledge as "Know how").

Along the continuum from data to information to knowledge (Fig. 1; *y'* axis on the left), the amount of human contribution, needed to perform the transition from a level to another, increases (Fig. 1; *y''* axis on the right). According with the literature (Rowley, 2007), data and information are characterized as phenomena in the universal domain, while knowledge as phenomena in the subjective domain. Consequently, the tools used to perform the shifts toward the knowledge (see paragraph 2.4) involved an increasing level of participation among actors, namely among the OR-Net (Fig. 1, *x* axis in the middle).

Finally, the participatory model involved important component of a feedback loop from the research to its outputs, since that the process of research was adjusted to produce more relevant and appropriate outputs (Lilja and Bellon, 2013). Therefore, the outcomes, at each level of the hierarchy, represented feedbacks, on the basis of which to adapt and re-design the tools and processes involved at all levels (Fig. 1, arrows of "feedback-and-adaptation cycle" on the left), in order to target the activities to the study goal. After the first year, the questionnaire for the interview was implemented with further open-ended questions and dialogue techniques more close to those used during the group fields monitoring. Starting from the results of the previous activities, the interviews shifted also in the "information' domain" of the DIKW pyramid, with the aim to capture information about the ORCS response to some elements highlighted as relevant. Similarly, after the first year, the system thinking, used during group fields monitoring and plenary meetings, capitalized the previous results, contributing to confirm or put in doubt the information and knowledge previously obtained.

As highlighted by other authors (Bruges and Smith, 2008; Berthet et al., 2016), the participatory approach is in allowing participants to further their goals as that themselves define them, while any limitation to the influence of participants in determining the goals risks limiting the method effectiveness. Therefore, this "feedback-and-adaptation cycle" pathway was applied with a certain extension also to the overall research process, i) leaving the farmer free to set the strategies to test for the ORCS during each growing season, ii) and giving space to the development of further studies targeting purposes beyond those shown in the present work. Thanks to this, other relevant issues have arisen and pointed out by the OR-Net, and consequently the OR-Net

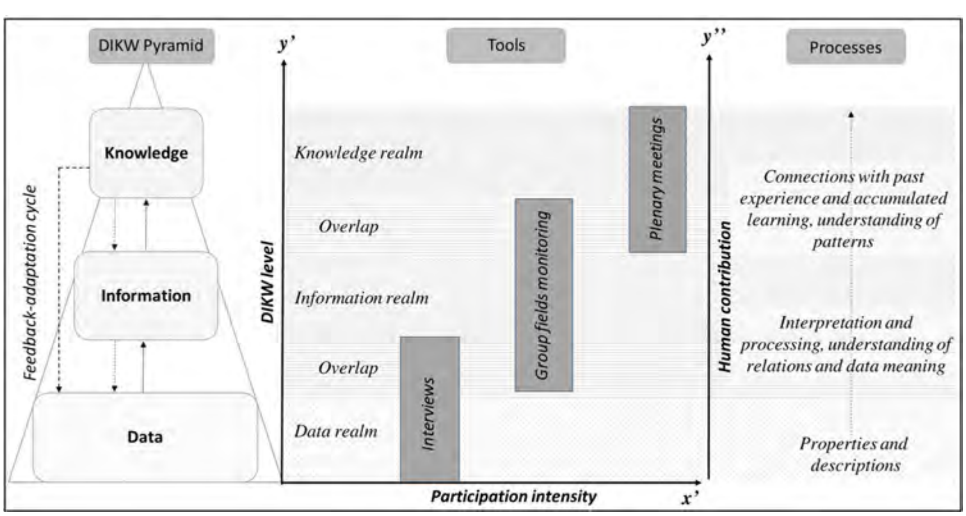

Fig. 1. Conceptual chart on the methodological approach: the DIKW Pyramid' levels (on the left); the corresponding processes to shift from a level to another (on the right); and the tools used in practice to perform the shift (in the middle). Legend: ☐= data realm; ☐= information realm; ☐= knowledge realm; ☐= overlapping, transition zone between levels.

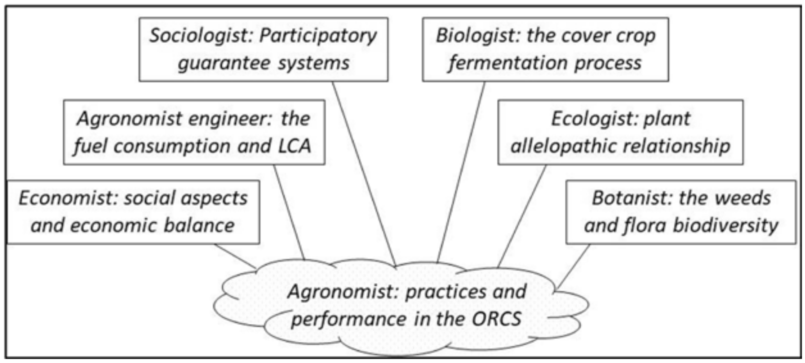

Fig. 2. The conceptual chart on the scientists involved into the OR-Net, in order to better deal with other relevant aspects for the ORCS, besides the core group of agronomists, focused on the management strategies and their performance.

incorporated new experts, approaching the interdisciplinary study of ORCSs, as shown in the conceptual chart of Fig. 2. The DIKW pyramid was implemented through practical discussions (see section 2.4) that occurred in interactive and informal environments. On-farm experiences were compared and explained, fostering the critical analysis and the ability to gain general principles from a wide range of case studies. This process occurred in horizontal and non-systematic way, giving space even to spontaneous extensions of further multidisciplinary researches and common actions, beyond the beginning goals.

2.4. The tools

Tools were used to realize a participatory system thinking that allowed to capture and validate in the research process the cumulative knowledge of the farmers, implementing the DIKW hierarchy and its key processes: the interpretation and understanding of the data meaning (toward information), and the linkages and integration with the past experience, accumulated learning, scientific literature, existing empirical and scientific knowledge (toward knowledge).

Interviews were conducted with the farmers in three phases of the growing season (i.e. at beginning, mid and end). The interviews were performed immediately after the application of the agronomic practices, to avoid the collection of incorrect or approximate information due to long time elapsing between the actualization of the practices and the interview. At least the first interview was conducted face-to-face and, depending on the complexity of the management strategy, the others were conducted using other communication channels (i.e. phone interviews, emails, messages via smartphone). Data were collected concerning: field size, tillage (e.g. machines, depth, schedule), irrigation (e.g. flooding depth, schedule), sowing (i.e. date, dose, variety, modality: depth or direct seeding, dry or water seeding, in-row or broadcast seeding), harvest (i.e. date, total harvest, grain moisture), and the crop residues management. The harvest data were converted into grain yield (t/ha at the commercial moisture, 14%). Data on the previous land use over the latest four years was collected (i.e. prior cover crop, year of set-aside with grass meadow or bare soil, crop rotation), as well as details on the cover crop management if there was (i.e. tillage, species, sowing), and related biomass management (i.e. lodged or shredded).

Group fields monitoring was carried out by groups of researchers and farmers (at least one researcher and one other farmer, besides the one who manage the farm), from two to three times per field, depending on the complexity and key periods of the management strategy. The field tour represented an opportunity of opinions comparison and participative brainstorming. The group members tried to find the nexus between yield, crop growth and weeds control (i.e. species' presence and incidence) on one hand, and the management or other element variables (i.e. soil, climate, etc.), on the other hand, sharing reflections, insights and suggestions (i.e. constraints of the chosen management, possible mistakes, corrective measures).

Plenary meetings were organized with the OR-Net members three times per year during the cropping season (i.e. at beginning, mid and end). One of the three annual meetings was extended also to other actors, inviting: i) experts with specific skills, useful to improve the ORCS understanding and knowledge (e.g. on compounds produced by the fermentation of cover crop, allelopathy between species, etc.) (Fig. 2), ii) other farmers, that shared their experience and were encouraged to join the OR-Net. During the plenary meetings the researchers schematically shown in presentations (i.e. PowerPoint) data and information related to each ORCS case study, also with the documentary support of videos and photos (all the materials were shared with the OR-Net through a folder on google drive). This encouraged the actors of OR-Net to share their expert opinions, performing the linkages between the collected data and information, and the existing wealth of experience and knowledge. Thanks to these focus groups over three years, the most promising managements were progressively characterized, identifying the functional principles underlying the strategies for weed control, and highlighting the positive and critical aspects, via SWOT analysis (Strengths, Weaknesses Opportunities and Threats). The productive responses of the ORCSs were analysed and shown by the researchers as quartiles (Q1 contains the lowest 25% of the data, Q2 in the median of the data and contains 50%, Q3 contains the upper 25% of the data), together with the descriptive statistic for each quartile (i.e. mean, standard error, standard deviation, variance). Then, the differences between ORCSs belonging to different quartiles were discussed, using dialogue techniques.

The *interviews* to the farmer were the main effort in *data* collecting, concerning the agricultural practices and the associated yield. The *group fields monitoring* was an opportunity mainly to enhance the interpretation of field data, with the understanding of the relationships between the ORCS performance and the agricultural practices or other environmental variables, producing *information* on the ORCS' responsiveness to the management choices. During the *plenary meetings* data and information were chorally discussed, operating the linkage with experiences and skills, and contrasting the opinions with the current scientific and empirical knowledge. This results in a progressive advancement in the state of *knowledge* of ORCS, and in the definition of successful management strategies and mistakes not to commit.

The responses chorally approved by the OR-Net were grouped according the potential causes of low yields, and in parallel, the key elements of successful decision making were pointed out. The researchers through careful listening, understanding and conversing with the OR-Net' members, learned the main sources of variability impacting the ORCS production. Table 3 shows exemplificative case studies of the ORCSs associated to the lowest yields of three farms. The table describes the process of analysis, based on the implementation of the DIKW framework that allowed to identify the variability sources and generate know-how among the farmers. "What data says": the data

Table 3
DIKW pyramid implementation, in order to identify the variability sources (VS) affecting the production of the less productive ORCSs belonging to three farms. Legend: CC = Flooding of green mulch from different cover crops; SD = Stale seedbed in dry paddy, in combination with comb harrow; cc = cover crop; A (i.e. Know-how), B (i.e. Optimization), C (i.e. Seed bank), D (i.e. Variety), E (i.e. Climate), F (i.e. Fertility), highlighted in the below example as among the causes of low yield in 6, 5, 3, 2, 2, 1, and cases out 9, respectively.

What the data says "management and yield"		*What the information says: "in what these fields differ?"*	*What the knowledge says: "conditions to avoid"*	*VS*
ORCSs with lower yield	***t/ha***	No plowing before cc sowing	Lack of plowing: [A)] negative impact on cc growth, and promotion	A
(< 3 t/ha)	[1]0.0	Little biomass developed by cc	of seed weed bank expression	C
- [1]CC strategy			Disadvantage conditions for cc growth: [B)] less is the cc biomass,	
- High weed incidence			less its effectiveness	
	[1]0.9	Pest attack (*Sitophilus oryzae*) promoted by rainy weather	Rainy weather, promoting pests	E
		No plowing before cc sowing	Lack of plowing: [A)] idem	A
		Little biomass grown from cc	Lack of rotation, promoting seed weed bank expression	C
		Rice cultivated for two years running		
	[1]2.8	Farm infrastructure shaded large part of the field	External factor, giving competitive advantage to weeds	
	[1]2.8	Earlier rice sowing	Early rice sowing, chopping the cc biomass before its full growth:	B
		Cc chopped before its full growth	[B)] idem	
ORCSs with lower yield	[2]0.5	Pest attack (*Magnaporthe oryzae*)	Variety susceptible to rice blast	D
(< 4 t/ha)		Rice variety different than usual	Excessively intense use of comb harrow after rice emergency	A
[1]CC and [2]SD strategies		Many and deeper passages of comb harrow after rice		B
Medium weed incidence		emergence		
	[1]3.2	Minimum tillage in previous two years	Lack of plowing, [A)] idem	A
				C
	[1]3.4	Previously the field was set-aside land with clover meadow	Excess of soil fertility, promoting excessive cc biomass growth: [C)]	F
		Great cc growth	resulting in too intense fermentation and rice damage	B
		Very intensive cc fermentation (water' red colour for organic	Inaccurate levelling with water stagnation: [C)] idem	
		acids concentration) in parts not well levelled		
ORCSs with lower yield	[2]0.7	Wheels' ruts by tractor passages	Inaccurate levelling, affecting comb harrow effectiveness	A
(< 4 t/ha)		Ineffective weeds' eradication by comb harrow		B
[2]SD strategy	[2]2.9	Rice sown deeper than usual	Sowing depth unsuitable for the soil texture: giving competitive	A
High weed incidence		Rice emergence not homogenous	advantage to weeds	B
		Pest attack (*Magnaporthe oryzae*)	Not pest-resistant variety in climatic seasons favourable for rice	E
			blast	D

describes for the ORCSs the management practices and the performance, in terms of weed incidence, and rice yield. "What the information says": the information gives answers to the question "what differentiates these ORCSs by the others with higher yield?", reflecting on the nexus between difference in performance and any other differing element. "What the knowledge says": the knowledge indicates the conditions ideally to avoid during the cultivation, in order to obtain better yields, namely the Know-How for organic rice farming.

The use of these tools was made effective by the active participation of the researcher in the farmer decision making process, and his/her involvement in the process of incremental and experiential learning through the adaptive management. This activity was time-consuming, with a periodic presence of the researcher in the farm life, in order to attend the management decisions during the key periods, actively monitor with the farmer and feedback, from the effects and outcomes of the decisions. Moreover, the farmers were interviewed by the same interviewer that also attended all the field group meetings and the plenary meetings. This because the understanding of the ORCS complexity required an overall vision, and then that at least one observer of the scientific community follow all the DIKW steps to recompose the general knowledge framework, as made with success by other authors in similar studies (Calheiros et al., 2000).

3. Results and discussions

3.1. The management strategies

Three promising management strategies for the ORCS were identified. They are not distinct by strict boundaries, since the adaptive management can lead to adopt a combination of more than one strategies in the same field, exploiting more than one functional principles for weed control. Each strategy does not represent a universal recipe, but it can be declined with a wide range of variants, maintaining the basing functional principles, but shaping the practices to the case specific needs.

What was observed in the present study is well commented by Bell et al. (2008): the organic farmers see their farming as something more than a process of recipe adoption. The real meaning of their management seems to be working with variation, across space and time, rather than against it, avoiding universal practices, proper of a reductionist approach: the farmer asks to agroecology help in identifying the driving ecological and agricultural principles, and, in the same time, space for her/his genius and contextual creativity.

The main agronomic practices and innovative aspects of each strategy are listed below.

Stale seedbed in dry paddy, in combination with comb harrow (SD). The strategy is based on the well-known technique of the stale seedbed (Ferrero, 2003). The weed germinated after the false seeding are removed with the comb harrow. The latter is used on dry soil, both before and after the rice sowing (i.e. 2–7 passages with fine spring tines; 3–4 cm of tillage depth). The effectiveness of comb harrow is conditioned by appropriate devices that allow to mechanically impact the weeds, without damage rice seedlings (e.g. suitable field levelling and regulation of tractor speed and tines' tillage depth to assure the uniformity and precision of comb harrow action; promptness intervention before the young weeds rooting). After the rice dry sowing (i.e. at 4–6 cm depth), the paddy field dry conditions are protracted (e.g. 20–30 days), in order to allow the comb harrow passages. The comb harrow is a novelty for the European rice production. Its use for rice is reported in less mechanized and low-industrialized agricultural contexts of Asian countries (i.e. studies talk about "indigenous" or "locally designed" comb harrows; Verma and Dewangan, 2006; Pande et al., 1994; Calilung, 1985).

Stale seedbed in flooded paddy, in combination with minimum tillage machines (SF). In this strategy, the stale seedbed is followed by the field' flooding, then the weeds eradication occurs in water through minimum tillage (i.e. 2–3 interspersed passages). For this operation the farmers use innovative machineries, realizing them by themselves, "ex novo" (e.g. “rotolama”, Valsesia et al., 2009), or modifying existing machines (e.g. adding tines to a bar normally used for field levelling). The management of flooding and the regulation of the water level need to be accurate, in order to ensure the removal through water of the eradicate weeds floating up to the surface. After the minimum tillage, the broadcast sowing of rice occurs. As the literature revealed, the weed control is performed not only mechanically, but also by the “puddling” effect: the tillage in saturated conditions destroys the top soil structure, and within the resulting mud, the weeds remains knead, finding anaerobic conditions, able to kill the young individuals and delay the seeds germination (Bhagat et al., 1996). The “puddling” technique is practiced in tropical agriculture of lowland rice, and effects in weeds’ reduction are reported (Ponnamperuma, 1981; Bhagat et al., 1996; Sharma and De Datta, 1985; Sureshkumar et al., 2016). However, the adoption of minimum tillage in this context is a novelty. In Asian agriculture the “puddling” is used with the main aim to reduce the percolation and softens the soil for rice transplanting, and it is realized through high energy intensive operations (i.e. deep tillage machineries: plough, harrow or rototiller).

Flooding of green mulch from different cover crops (CC). A cover crop is introduced before rice (e.g. *Lolium multiflorum* Lam.). The rice is sown on the cover crop plants. Then, the cover crop is chopped, lodged or left standing, and flooded, activating an anaerobic fermentation of its biomass. The fermentation has negative impacts on weeds germination, and, to a lesser extent, also on rice. Therefore, fermentation’ intensity and duration are key aspects, controlled by many factors (e.g. temperature, biomass quantity). Many agronomic variants are tested by the farmers in order to increase the weed control and decrease the impact on rice. Mainly the farmers tested two different pathways: the broadcast sowing of rice, immediately followed by flooding (i.e. the cover crop fermentation occurs at rice germination growth stage); or the rice dry sowing, followed by a protracted dry conditions (e.g. 20–30 days) (i.e. the flooding and fermentation occur at rice leaf development growth stage 3°- 4° leaves unfolded). The CC strategy is completely innovative and there are no references in literature concerning its use. It is based on complex dynamics and requires further studies (e.g. competitiveness and allopathic relationships between weeds and cover crop species, green mulching effect, weeds and rice susceptibility to the organic acids of the fermentation, etc.). This strategy was evaluated by the OR-Net as the most promising, since it showed wide room for improvement and is in line with the farmers’ agroecological vision.

The underlying techniques and functional principles of each management strategy, and the results of the SWOT analysis are summarized in Table 4.

3.2. The productive performance

The yield of the “ORCSs population” showed a normal distribution (Skewness = - 0.33; Kurtosis = - 0.01) (Fig. 3; Fig. 1a and b in supplementary material), and high variability, ranging from 0.0 t/ha (i.e. harvest failed) to 7.1 t/ha, with: mean of 3.7 t/ha, median almost 4 t/ha, standard error of 0.23, standard deviation of 1.6, and variance of 2.6 (Fig. 4) (Table 1a and 1b in supplementary material). Table 5 shows the mean and different measures of variability for the three yield’ quartiles, while the box plots of Fig. 5 show for each of them the area within which 50% of the data are distributed and the position of the median.

The highest variance was found for the lower quartile Q1, followed by the upper quartile Q3, highlighting the wide yield fluctuations associated with the unsuccessful production (i.e. Q1), as well with the greatest productive performances (i.e. Q3), while the medium quartile Q2 shows a certain yield stability and data homogeneity.

Concerning the Q1, the OR-net has identified, firstly, the weed competition, promoted by management errors (i.e. strategy or technique unsuitable for the cultivation condition, lacking punctuality and timely of the interventions), as the main cause of the yield decline, in

Table 4
Features of the tested on-farm management strategies for weed control: techniques, functional principles, and results from the SWOT analysis. Legend: TEC = Techniques; FP = Functional principles.

Strategy	*Strengths*	*Weaknesses*	*Opportunities*	*Threats*
TEC: SD (Stale seedbed in dry paddy, accompanied with comb harrow); **FP**: Mechanical weeding	• Suitable also in case of intermittent water access • Easy to learn (short-time to build up the required skills) • Available know-how (better-known technique) • Low yield variability	• Not suitable for firm soil • Postpones the rice sowing for mechanical passages • Many tractor passages	• Comb harrow can be used to organically grow other crops	• Operations are hampered by rainy weather with risk of excessive sowing postponing
TEC: (SF) Stale seedbed in flooded paddy, accompanied with minimum tillage; **FP**: Mechanical weeding, puddling effect	• Few tractor passages • Not affect the rice sowing date • Not hampered by rain	• Not suitable for loose soils • Needs flexible access to water • The machine is not versatile for other crops • The machines development and use are at earlier experimental phase • Slow know-how generation (least used) • High yield variability	• Rescue measure in case of need timely rice sowing • Possibility to attract private investments in mechanical innovation	• Farmer invests in a machine, without certainties on its suitability to the site-specific features • The scarce adoption can lead longer time for its improvement
TEC: (CC) Flooding of green mulch from cover crops; **FP**: Competitive effect of cover crop and green mulching, chemical control (e.g. organic acids, allopathic compounds)	• Low number of tractor passages • No involves economic investment in machines • Shows highest productive potential • Range of variants available to shaping a site-specific solution • Fast know-how generation (most used)	• Not suitable for loose soils with fast drainage • Rotation plan can encounter the cover crop sowing • Requires a careful and timely management • Needs flexible access to water • Effectiveness affected by weather and very dependent on experience (lack of unique recipes and structured know-how) • Higher yield variability	• Basing on endogenous elements, instead of mechanical input, promotes agroecological models • The bottom-up innovations progressively minimize the disadvantages • Some FPs can be transferred to other crops • Its low-input nature (no investments) incentives new farmers to try the organic farming • the incomplete understanding of its FPs leaves space for researches development	• Being characterized by a high level of unpredictability and responsiveness to small changes, involves wide risks of harvest losses

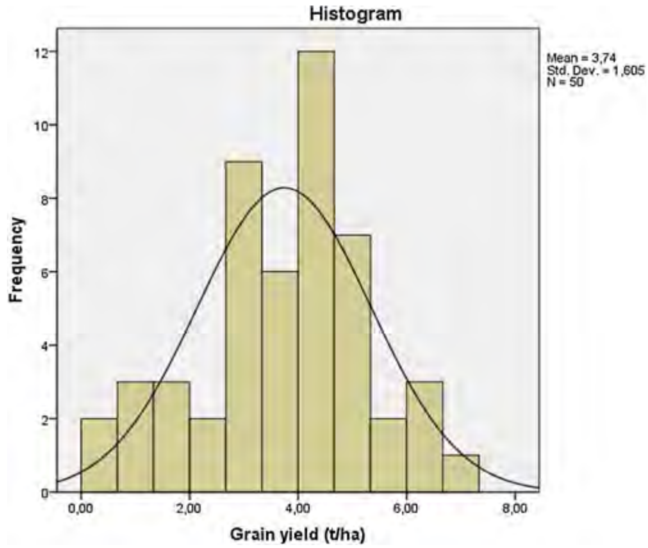

Fig. 3. Normal distribution of frequency for ORCS yield.

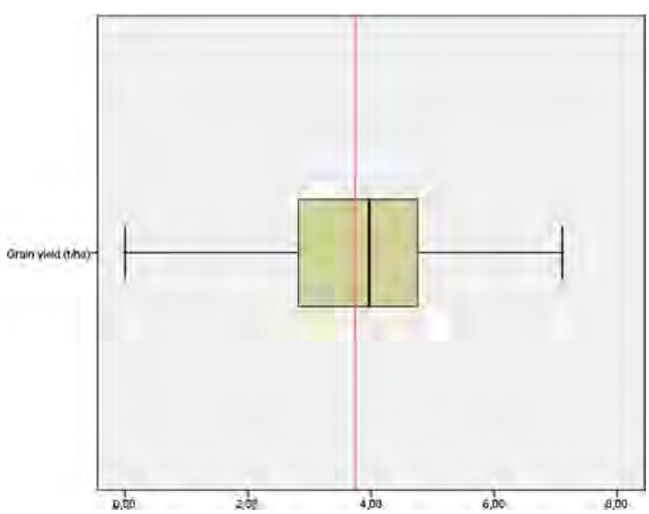

Fig. 4. Graphical representation of the descriptive statistics on ORCS yield. Legend: box borders = thresholds of Q2, containing 50% of the data; horizontal bars = max. and min. values; vertical out box line = mean value; vertical in box line = median value. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 5
The descriptive statistics for the three quartiles of yield (t/ha) for the ORCS.

Quartile	Mean	Std. Deviation	Median	Variance
Q1	1.67	0.98	1.88	0.97
Q2	3.91	0.57	4.00	0.32
Q3	5.65	0.77	5.37	0.60

accordance with the literature (Delmotte et al.;, 2011; Shennan et al., 2017; Hazra et al., 2018), and secondarily, the pests attack (i.e. water weevil, *Lissorhoptrus oryzophilus*; rice blast, *Pyricularia oryzae*), promoted by the weather conditions and susceptible varieties. Concerning the middle quartile Q2, the median yield was 4 t/ha, and its position, far from the centre of the box plot (Fig. 5), indicates a tendency of the values to be close to the upper threshold, while a few data have contributed to decrease the bottom threshold, and thus the mean. These yields are in line with the productivity of organic rice recorded in the European countries (i.e. without the use of hand weeding): Bacenetti et al. (2016) showed an average yield of 4.5 t/ha in a farm located in

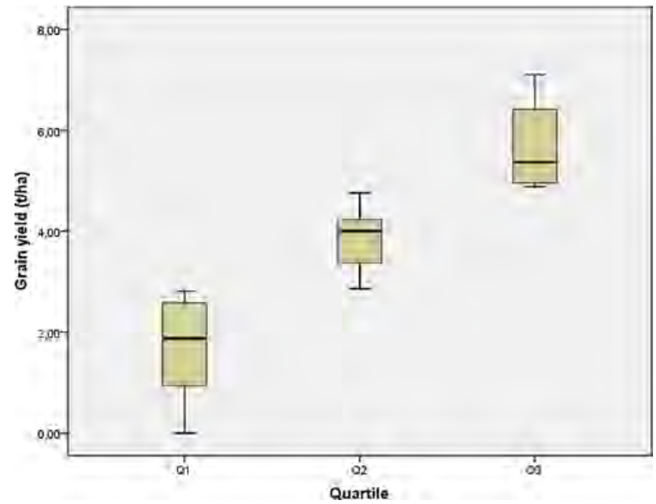

Fig. 5. The box plot visualized for each quartile of ORCS' yield.

the same Italian rice area, and Delmotte et al. (2011) found on average 4.3 t/ha by analysing an extensive database for similar rice district in France (i.e. Camargue). Regarding the upper quartile Q3, the position of the median (Fig. 5), far from the centre of the box plot, indicates the tendency of the included data to be close to the bottom threshold of the box, while few data contribute to increase the top threshold, and thus the mean.

The highest yields of Q3 (e.g. 6–7 t/ha) were close to those observed for conventional rice in the same area (i.e. 6.8 t/ha; Bacenetti et al., 2016), and in line with those shown for organic rice in China, where the weeds competition was almost totally controlled with hand weeding: Huang et al. (2016) and He et al. (2018) report average yields of 6.1 t/ha and 6.3 t/ha (grain moisture at 14%), respectively. The highest yield values observed in this study give an idea about the potential production that could be reached by the ORCS, opening up the prospect that organic rice of European countries could reach in the long-term a productivity similar to conventional systems or to the Asian organic systems based on hand weeding.

Finally, the results invite the scientific community to consider the ORCS as a farming system that is evolving over the time, under the ongoing pressure of the bottom-up innovations and know-how generation among farmers' community, and then to not evaluate the ORCSs as a group of unchanging and static systems. The researchers that carry out environmental (e.g. LCA, carbon or water footprint) or economic evaluations in this area of study, with different outcomes depending on the agronomic inputs and the harvest per hectare, should become aware about the existing heterogeneity within the ensemble of ORCSs. Concerning this, different scenarios with a range of possible results should be implemented, basing on the wide range of management options, as farmer response to the time- and space- variability (see section 3.1), and the harvest fluctuations.

3.3. The variability sources affecting the yield

The identification of the factors that determine yield variability and productivity gaps between organic and conventional farming is an important step towards the design of more ecologically intensive rice cropping systems (Delmotte et al., 2011). Concerning this, the obtained results allowed to understand the large yield variability observed among the ORCSs (see Section 3.2), identifying the main variability sources (VS) affecting the production. The progressive implementation of the DIKW framework during the three-year participatory study led to highlight the causes underlying the best and worst yields and show the ORCS as a complex and difficult to predict system, whose performance depends on many interacting and interconnected VS, as described below.

The know-how (A): the main factor affecting the ORCS performance was identified in the farmer skills, i.e. in capability to develop know-how about the ORCSs. An agronomic solution cannot be successfully repeatable in any productive context, since its feasibility is conditioned by site-specific features (e.g. water supply, soil hydrology and texture; Table 4). The farmer capability to understand the peculiarities and needs of the cultivation environment and, on these bases, to identify ad-hoc agronomic solutions (e.g. choose, adapt, eventually modify or invent context-specific practices) is currently the key element to reach satisfactory yields. The know-how depends on the direct experience of the farmers, and then on their capacity to learn through the adaptive management, using creativeness, intuitive ability and improvisation skills to address the uncertainty. The know-how also depends on the indirect experiences, transmitted by other farmers, and then on the farmer inclusion into a system of knowledge sharing within the rural community. The farms' distribution in Q3 (upper quartile of yield; Tab. 1b in supplementary material) confirms the influence of the farmer know-how on the ORCS' productivity: the farm ID2, characterized by a long-term experience in organic agriculture and led by one of the farmers' leader of the OR-Net, is present five times out of 12.

The optimization (B). After the identification of the suitable management techniques (A), these need to be applied by the farmer with the required accuracy. The ORCS performance showed high susceptibility to small changes in the timely and punctuality of the operations. Then, the agronomic practices need to be optimized in time and in space, doing the right thing at the right time and in the right place. For example, small changes in the tillage' depth and dates, in the flooding time and duration, in the soil levelling accuracy, can compromise the successful of a suitable strategy, hampering its effectiveness and the functionality of the underlying agronomic principles working in weed control.

The seed bank (C). The expression of the soil weed seed bank depends on the dynamics of the annual plants population, which is complex and difficult to predict (Borgy et al., 2015). It depends on many factors, many of which in turn are included in the other VSs (e.g. management' effectiveness: A, B; soil and climate' conditions: E, F). Then, the VS, here defined "Seed bank", concerns the historical causalities of the field that, beyond the current rice growing season, promote or not the weed development. The seed bank expression depends on the endemic characteristics of the agroecosystem, the previous land use (e.g. crop rotation, set-aside), and the past operations (e.g. tillage). The negative impact, that was observed associated to the continuous cultivation of rice (for more than two or three years) or to the lack of ploughing in the past seasons (minimum tillage), pointed out the key role of the long-term effects in the complex environment of the ORCS.

The variety (D). The variety affects the yield in different ways, also on the basis of the interaction between the genotype' traits and the other VSs: taller varieties can show higher competitiveness with the weeds, short-cycle varieties better fit with management strategies that involve rice sowing delays, genotypes with higher potential yield lead to better harvest in favourable weather seasons, while with climate favourable to pest attacks show lower yield than that obtained with low-yielding but pest resistant varieties.

The climate (E). The climate and the weather trend affect the rice grow and the incidence of biotic (e.g. pest) and abiotic (e.g. thermal stress) damages, and impact the effectiveness and scheduling of some management operations (Table 4). Beyond the annual trend, a substantial difference in productivity was observed between the fields in Vercelli and Pavia Province. The coldest agro-environment of Vercelli results in slower rice growth and lower production. This condition is exacerbated by the closeness of the rice fields to the water sources (i.e. the fields close to the source get colder water, the latter suffers a gradual warming while reaching the farther fields through a system of watercourses and channels).

The fertility (F). Compared to the conventional farming, the soil fertility affects the ORCS yield with some peculiarities. The fertility of organic soils depends on the long-term and cumulative processes (i.e. increase of humus and biological fertility through crop rotations, green mulching, green manures), rather than to the fast action of input, and its effect on the yield is less predictable. Some examples: a good nutritional status can promote the rice growth, as well as lead to an excessive growth of the cover crop or the weeds, with consequences not always positive for the harvest.

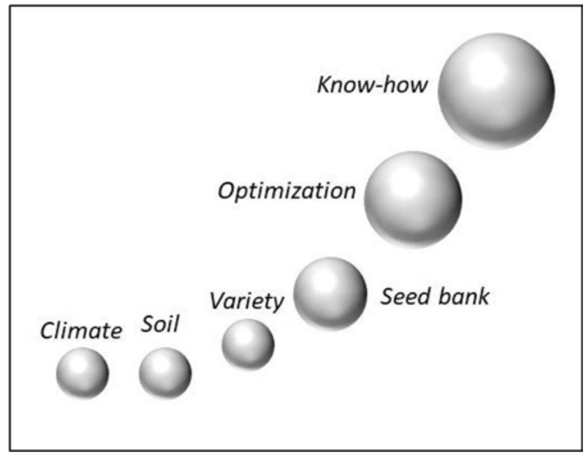

Fig. 6. Conceptual chart describing the current weight of the variability sources driving and affecting the productive performance of the ORCS.

Fig. 6 is a conceptual representation of the relevance currently evaluated for each VS. It considers the lowest quartile (Q1), for which A, B and C were identified as responsible of the low yield in 77%, 54% and 31% of the cases, respectively, while D, E and F were among the causes of the low production in only about 15% of the cases.

These results suggest that, at the early development of organic rice farming, soil (F), climate (E) and variety (D), that usually play a key role in determining the conventional farming performance, cover a secondary role instead. Their effects on yield are mostly hidden by the strong impact of the other VSs mainly related with the field management and its effectiveness on weed control: the suitability of the chosen strategy and agronomic solutions (A), the optimal application of the practices (B), the past operations and land uses (C). The participatory study showed as, in the beginning of the Italian rice sector, these VSs are predominant in driving and affecting the performance of the ORCS.

These results are confirmed by the inter-annual average trend of yield observed for the ORCSs. Thanks to the improvement over time in the farmer' know-how, management skills and techniques refining, an increasing average yield, accompanied by a decreasing standard deviation were observed. Compared to 2016 (mean = 2.85 t/ha, standard deviation = 1.77) the average yield of the ORCSs showed an increase of 34.7% in 2017 (mean = 3.84 t/ha, standard deviation = 1.47), and of 61.6% in 2018 (mean = 4.61, standard deviation = 1.13). On the other hand, the trend of conventional rice yield, reported by the national statistics (ISTAT, 2016-2018) in the study area (Vercelli and Pavia Provinces), showed a quite stable production, with average yield ranging between 6.9 (in 2016 and 2017) and 6.6 (in 2018). This suggest that the increasing productivity, observed in the ORCSs during the three years, is related to dynamics not in common with the other farming systems (i.e. preponderance of A, B, and C impacts, instead of those of D, E and F).

The results suggest the crucial role of the bottom-up innovation and the knowledge-intensive agronomic practices development for improving the management and yield in the ORCS. These processes were supported by the knowledge sharing among the OR-Net' members. The three years of participatory study led to a better understanding and farmers' awareness about the relationships between the management choices and the ORCS yields, toward an overall improvements of the performance.

In this context, it is reasonable assume that the current framework about the variability sources weight is only temporary. Further developments in innovation, know-how, and management skills, can lead to minimize the impact due to A, B and C, a then to a progressive increase of the relative weight of D, E and F. This in the future, can lead to reshape the frequencies distribution' curve for the yield shown in Fig. 3, shifting it forward (i.e. increase of mean yield) and shrinking its width (i.e. decrease of yield variability).

3.4. Reflections on the participatory approach

A knowledge-intensive and qualitative research methodology was applied, mainly based on researcher-farmer dialogue techniques and a collective system thinking among different actors. This turned out to be a useful approach for studying complex systems, when the study is in the early stage and starts from poor background knowledge. The participatory study allowed to make sense of collected data through the experience of the farmers, overcoming the gap between scientific and empirical knowledge. Tangible improvements occurred in the state of knowledge on the ORCS, its management practices, inputs, and performance. These results will be useful to drive further scientific inquiry and evaluations. Others positive externalities of the participatory research were observed:

- It supported the spreading and sharing among the farmers' community of agroecological principles and flexible agronomic solutions, instead of unsuitable universal receipts. As consequences the farmers' know-how and organic rice yield increased.
- it promoted the connections between the farmers' and scientific community (see Fig. 2) giving space to further research extensions, targeting the sector needs. Currently, following farmers' advices, specific studies are ongoing on the allopathic relationships between weeds and crops, and on all-encompass environmental evaluations (i.e. GHG emissions balance, flora and soil biodiversity)
- Above all else, it generated social innovation. Meetings and interactions among the OR-Net members, and the three years' collaboration for a common aim fostered trust relationships, and the social cohesion among farmers. As result, in the early 2019, the farmers have founded a group of producers legally recognized: the farms' network "Noi Amici della Terra" (means "We Friends of Earth"). This is an aspect extremely innovative for the Italian rice sector that lacks of any aggregation forms among producers. The farms' network allowed to build a group entity in the market, facilitating the scouting of new sales channels and advantageous agreements, strengthening the farmers' power in the food supply chains.
- It has built a system of mutual-learning, knowledge sharing and coaction, that is continuing to exist, and is independently implemented by the farmers with exchanges of news and information (e.g. suggestions, field pics, discussion the techniques within animate whatsapp OR-Net group), and coordinated actions to address specific issues (e.g. work tables on economic or legislative issues).

On the other hand, the applied methodology was also characterized by constraints and weakness. The DIKW pyramid implementation mainly occurred in a horizontal and non-systematic way, despite the attempt, since the beginning, to follow an organized scheme and timetable for the ORCS monitoring. Data, information and knowledge were obtained in many cases following a flexible path, conditioned by the farmer' timing and based on the use of different supports for the farmers' communications (e.g. updates sent by farmer with notes, pictures or videos via email or whatsapp, face-to-face dialogues, questionnaires form, phone calls, etc.) and the researcher' documentation (e.g. writing on field notebooks, voice recordings, photos, videos, etc.). Then, the subsequent effort in synthesis and in organizing all the resulting data, information and knowledge was great and time-consuming.

Moreover, the researcher had to spend long time living the reality of the farm, constructing a common agronomic vocabulary with the farmers (i.e. "talk the same language"), dialoguing with them, participating in the decision making processes. In this context, the reaching of a systemic vision of the ORCS and its dynamics, is similar to compose a puzzle. At least one researcher had to follow all the steps involved in the DIKW pyramid, in order to track, reorder and understand the "pieces", obtaining an overview (i.e. to study the puzzle' pieces and then compose the whole framework). This results in a limited possibility to alternate the researchers in the activities, with the risk to overloading a single person, especially during the overlapping of the fields monitoring in the key periods for the ORCS. On the other hand, the possibility that two researchers can monitor together all the ORCSs, mutual-helping each other, is constraints by the excessive effort in terms of human resources.

Finally, the successful of the participatory research was conditioned by the presence of some preconditions (i.e. matching of farmer and researcher needs and wills; see section 1.3), and by the availability of farmers, that share ethical values and collaborative spirit, and researchers able to strength skills in social relations (e.g. approaching farmers through peer-to-peer relationships and learning that it is an important way to gain their respect and trust). Then, the human component intrinsically present in the participatory research adds elements of uncertainty. In other words, the results obtained applying the same methodology to other case studies could be different, affected by the partially unpredictable evolving of human interactions and personal expertise in this field.

4. Conclusion

The study proposed a knowledge-intensive and qualitative research methodology based on the participatory approach between researcher-farmer and on the integration between scientific and empirical knowledge to fill the knowledge gap about organic rice, which is resulted from the current reductionist research approach uncomfortable in studying complex agroecological systems.

The research led to an improvement in the state of knowledge about the organic rice, describing the performance and the main variability sources affecting the production at the early stage of organic agriculture development in the Italian rice sector. The research also identified the agronomic principles, practices and innovative aspects of the successful management strategies resulting from the bottom up innovation process led by a small group of pioneers. The results will be useful to make less uncertain the challenge of the transition toward organic agriculture, supporting the conversion of conventional farmers, in a hardly encouraging rural context, where high-input and mono-cropping systems prevailed. Moreover, the results will provide to the scientific community a framework on the ORCS useful to drive further scientific inquiries and evaluations coherently with the reality faced by the farmers, and then taking into account the wide variability that characterizes the management and the in time evolving of the productivity, under the pressures of the know-how and innovation generation. The participatory research showed also the capability to generate social innovation, leading to the creation of an innovative form of aggregation among organic rice producers that was based on the peer-to-peer and trust relationships established during the three years' study. However, even if the methodology proved to be useful for an explorative study of unknown complex system, the revealed weakness highlighted the need to make more systematic and schematic the data, information and knowledge collection and to integrate the present results with further study performed with the traditional research approach. Moreover, the study pointed out as the main constraints of the participatory approach the time-consuming of the research activities and to the unpredictable component related to the human contribution, intrinsically belonging to this type of approach.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agsy.2019.102739.

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Chapter II

In allelopathy processes, it was found that some crop residues can partially suppress the presence of weeds, thanks to the release of compounds with inhibitory and phytotoxic, and therefore allelochemical, action. Some researchers have experimented with different types of grain residue management, including incorporation into the soil or burning of the same, but the use of straw as mulch has given the best results in terms of weed quantity and final rice yield but also owned by the soil (Khaliq et al., 2013). However, it must be taken into account that the suppressive action of crop residues can also affect the growth of the rice itself. Field tests have been carried out in Cambodian territory, demonstrating this drawback. The residues of some rice varieties incorporated into the soil have shown the ability to interfere with weeds and rice development. The minimal or no effects on this were obtained only by incorporating crop residues in smaller proportions or extending the period between adding residues to the soil and sowing rice (Pheng et al., 2010). The limits of this practice are represented by the limited or difficult availability of vegetable residues and by the fact that rice straw, for example, has a certain economic value as it is used in animal husbandry or as fuel (Rodenburg & Johhson, 2009). The mulching effect is one of the mechanisms offered by cover crops in green mulching. The study Fogliatto et al. 2021 was carried out in 2017 and 2018 regarding this technique in Italian farms located in Rovasenda and Livorno Ferraris, in the province of Vercelli. In the experimental areas, the main weeds were crodo rice (red rice), E. crus-galli, C. difformis and H. reniformis. The main species of the study were mainly Lolium multiflorum (ryegrass or ryegrass) and Vicia villosa (hairy vetch), which were sown as a cover crop, thus occupying the ground in the autumn-winter period before the spring sowing of the rice. This technique has been observed to reduce the presence of weeds thanks to the four fundamental mechanisms previously described: greater competitiveness of cover crops, mulching effect, allelopathic effect, and production of phytotoxic compounds resulting from fermentation in the water of the paddy field. The best suppression of weeds was achieved with ryegrass and with the ryegrass mixture at 60% and vetch at 40% in Rovasenda in 2018.

Furthermore, the weed abatement technique (rolling or shredding) did not significantly differ in the quantity of weeds and yield. The study has shown that ryegrass is more resistant to the rigours of winter and no-till seeding, unlike vetch, which is less tolerant of low temperatures and thrives in well-worked soils. Therefore, it is necessary to consider the climate of one's cultivation area to ensure good emergence and growth in biomass of the cover crop to better favour the mechanisms described.

Chapter II focuses on evaluating the allelopathic effect of cover crops in the management of rice weeds.

The study began from the curiosities about the agronomic techniques described in chapter 1. In particular, reference is made to the green mulching technique (named CC in the previous Chapter). Several tests in the growth chamber allowed us to evaluate whether there was a competitive effect of cover crop and green mulching for chemical control (e.g. organic acids, allelopathic compounds) against the most famous rice weeds. The methodology of the work and the results are presented in two publications, shown below.

This work was an opportunity to tackle bibliographic research on the allelopathic potential that some rice varieties intrinsically possess during the years of study. From this bibliographic study, a search emerged through world germplasm banks to obtain allelopathic rice samples. I requested 18 accessions of rice from the bibliography that had an allelopathic effect and could be of potential interest for further studies.

During the last year of my PhD, I co-supervised a three-year trainee for: - bibliographic research of the main allelopathic varieties worldwide; - cultivating in mesocosms 18 accessions of allelopathic rice to draw up morpho-physiological datasheets of the plants most suitable for growth in Italy, according to the standard used by Ente Nazionale Risi.

A further related study that is still being developed through a three-year internship concerns evaluating the chemical-physical characteristics of the fermentation waters of the cover crop and the possible competition effect on weeds correlating results emerged from flora surveys and rice yield data.

plants

Article

Potential Role of *Lolium multiflorum* Lam. in the Management of Rice Weeds

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Abstract: The phytotoxic relationships between crops and weeds can cover a role in weed management, reducing the use of chemical herbicides. Starting from the organic farmers' experience, the study aimed to define the inhibitory action of *Lolium multiflorum* Lam., used as a cover crop before rice sowing, against *Echinochloa oryzoides* (Ard.) Fritsch, one of the main rice weeds. In vitro 7-day assays were carried out in Petri dishes to compare the effect of different *L. multiflorum* Lam. parts, in the form of aqueous extract or powder, on the seed germination and seedling growth of *Oryza sativa* L. and *E. oryzoides* and to verify the hypothesis of a higher susceptibility of the weed. The total polyphenolic content, as the potential source of allelochemicals, in the *L. multiflorum* parts was measured. The results showed that both species suffer the phytotoxic action of *L. multiflorum*, but a more marked effect against *E. oryzoides* was recorded. In according with the polyphenol quantities, stem and inflorescence extracts showed the more significant species-specific inhibition. In all assays, the weed showed a stronger reduction in the root length and seedling vigor index, and, in some cases, also in the germination percentage and shoot length compared to rice.

Keywords: Italian ryegrass; barnyard grass; rice; cover crops; organic farming; weed control; phytotoxic activity

1. Introduction

Weeds cause severe crop losses in rice production worldwide. The yield reductions in flooded paddy fields are due to the presence of invasive aquatic and semi-aquatic species. Among them, the species of the *Echinochloa* genus are the most common weeds in wetlands and water-saturated conditions and are included in the list of the ten worst weeds in the world [1]. In particular, *E. oryzoides* (Ard.) Fritsch, known as early watergrass, is a rice mimic with very similar emergence and flowering times that allow it to achieve greater competitiveness than other *Echinochloa* species by influencing rice in its early growth stages [2,3]. Therefore, any intervention able to control the incidence of this weed is useful to give rice a competitive advantage.

Rice growers in temperate regions (Europe, US, Australia) are particularly attentive to weed control. Within an established model of industrial agriculture, based on monoculture and high-input systems, they face no option other than the application of synthetic herbicides because of the low knowledge of alternative agronomic practices and plant-based solutions, as well as the unfeasibility of hand-weeding due to the high labor costs [4]. However, the herbicide-based weed management has proved to be unable to solve the issues, leading to well documented resistance phenomena as in the case of *E. oryzoides* [2,5,6].

In addition, the special attention of the European Union to the risks and hazards for humans, animals and the environment associated with the use of chemical substances has led to the banning in its member states of many herbicides, such as oxadiazon-based plant protection products, a compound largely used in rice fields [7–9].

Given the above, there is the need to move toward new and more sustainable weed management strategies [10,11].

In this context, the long-term experience of the organic farmers could be used to recognize and set innovative and good practices, transferable somehow also to the conventional or integrated systems [12,13]. They paid particular attention to solve the weed issue identified as the main cause of yield variability and loss in the Mediterranean regions, which is the main challenge for organic rice production [14,15].

Weed control in organic farming is carried out through crop rotation and the use of cover crops, smother crops and green mulching, which are important for regulating the weed seed population in the soil and the plant population in the field [16,17]. In this regard, several factors influence the weed growth such as competition for space, water and nutrients, changes in temperature and shade as well as toxic microbial products, soil pH and release of allelochemicals [18]. Especially allelopathy, defined as the release of compounds from the living or dead tissues of a plant species with strong phytotoxic effect towards another one, is a phenomenon which deserves attention in the study of alternative pest management options, thanks to its potential action in weed suppression [19,20]. It is known that some species or varieties, used as cover crop or crop in rotation, produce relevant amounts of allelochemicals significantly affecting the weed germination and growth [21].

Allelopathic compounds could be used to control weeds in both organic and conventional agriculture: in the first case, through the direct cultivation of allelopathic species, respecting the ban on the use of any products for herbicide purposes and with the principle of low-external-input farming [22]; while, in the second case, through the marketing and use of plant-based herbicides able to support and integrate weed management, reducing the need for synthetic herbicides.

Particularly for rice, a participatory research carried out by Orlando and co-workers [23] with a small group of organic farmers in North Italy identified a strategy based on the cultivation of *Lolium multiflorum* Lam. as the most promising practice for weed control. In the study area (Po Valley, between Piedmont and Lombardy regions), characterized by a typical Mediterranean climate, 94% of national rice production is concentrated. The farming systems are mainly based on continuous flooding and a wide use of pesticides and herbicides that caused the highest groundwater and surface water pollution in the country [24]. *L. multiflorum*, known as Italian ryegrass, is used by rice growers during the winter season as a cover crop. Then, in May, the rice is sown directly among the standing plants of *L. multiflorum* and, subsequently, its biomass is mowed, chopped or rolled, producing green mulch. The farmer's empirical knowledge suggested to the researchers the existence of an allelopathic suppressive action of *L. multiflorum* versus *Echinochloa* spp., with a chemical inhibition of weed germination and growth, beyond the well-known competitive effect of green mulching.

Accordingly, the present study was aimed to verify the inhibitory activity of different organs of *L. multiflorum* against *E. oryzoides*. Two in vitro bioassays were carried out in order to evaluate the possible release of phytotoxic chemicals from the cover crop separately from other factors occurring simultaneously in the field able to influence the weed growth and from the complex dynamics of the soil seed bank. The impact of the *L. multiflorum* biomass aqueous extracts and its powder was assessed versus the germination and seedling growth of both *E. oryzoides* and *O. sativa* to highlight a potential species-specific action of *L. multiflorum*.

2. Results

2.1. Stem Effects

The obtained data showed a significant impact of the *L. multiflorum* stem aqueous extract on all the considered indices, except for the mean germination time (MGT), in both target species, but with a more evident effect against *E. oryzoides* than *O. sativa*, (*p*-values ≤ 0.05 for the interaction "species × *L. multiflorum* stem extract treatment") (Table 1).

Table 1. Germination indices measured for *E. oryzoides* and *O. sativa* under the effect of different concentrations of *L. multiflorum* stem extract.

Species	Stem Extract Concentration (%)	Germination (%)	MGT	Root Length (mm)	Shoot Length (mm)	SVI
E. oryzoides	0	100.0 ± 0.0 a	5.0 ± 0.0	56.2 ± 14.5 a	34.0 ± 3.9 a	9008 ± 1764 a
	1	83.2 ± 13.6 ab	5.8 ± 0.4	64.0 ± 4.0 a	28.0 ± 4.0 ab	6613 ± 657 b
	10	76.8 ± 17.4 ab	5.6 ± 0.5	62.0 ± 4.0 a	26.7 ± 0.6 b	5323 ± 227 b
	20	50.0 ± 35.6 bc	5.8 ± 0.5	58.0 ± 0.0 a	26.0 ± 0.0 b	2787 ± 0 c
	50	22.0 ± 22.8 c	5.0 ± 0.0	9.7 ± 6.7 b	22.0 ± 2.6 b	1198 ± 647 c
	100	20.0 ± 12.2 c	5.5 ± 0.6	4.0 ± 2.2 b	14.5 ± 1.0 c	482 ± 21 c
	F	13.756	2.892	36.382	22.701	43.232
	p-value	0.000 *	0.052	0.000 *	0.000 *	0.000 *
O. sativa	0	96.8 ± 4.6 a	5.0 ± 0.0	43.0± 13.5 a	19.8 ± 2.6 a	6072 ± 1525 a
	1	96.6 ± 3.5 a	5.0 ± 0.0	49.0 ± 1.0 a	19.0 ± 2.0 a	6364 ± 276 a
	10	96.6 ± 3.5 a	5.0 ± 0.0	52.0 ± 3.0 a	18.3 ± 5.5 a	6805 ± 1009 a
	20	96.6 ± 3.5 a	5.0 ± 0.7	47.0 ± 2.0 a	15.7 ± 1.5 ab	6042 ± 149 a
	50	79.0 ± 12.1 a	5.0 ± 0.0	20.2 ± 10.8 b	11.0 ± 1.6 bc	2564 ± 1235 b
	100	61.6 ± 21.1 b	5.2 ± 0.4	8.6 ± 2.1 b	8.6 ± 1.1 c	1003 ± 403 b
	F	9.922	0.286	19.413	15.045	24.718
	p-value	0.000 *	0.916	0.000 *	0.000 *	0.000 *
Interaction species × treatment						
	F	6.709	1.396	2.999	2.513	8.130
	p-value	0.000 *	0.252	0.025 *	0.05	0.000 *

Values are mean ± standard deviation, asterisk and different letters indicate statistically significant differences at *p*-value ≤ 0.05 among treatments in each species. F-value and *p*-value of the ANOVA test. MGT, mean germination time; SVI, seedling vigor index.

In particular, the extract, from 20% to 100% concentration, significantly reduced the *E. oryzoides* germination percentage (by 50%–80%), while *O. sativa* germination was affected only by 100% extract concentration with a 36.4% decrease compared to the control. Moreover, stem extract was able to inhibit *E. oryzoides* root and shoot elongation (up to 93% and 57%, respectively) by significantly lowering the seedling vigour index (SVI) values for all used concentrations (*p*-value = 0.000). Otherwise, only the treatments with 50% and 100% extract concentrations were effective on *O. sativa* whose SVI, root and shoot length were reduced by 58%–83%, 53%–80% and 44%–57%, respectively.

Bioassay carried out with stem powder provided less evident effects (Table 2).

Table 2. Germination indices measured for *E. oryzoides* and *O. sativa* under the effect of different quantity of *L. multiflorum* powdered stems.

Species	Powdered Stem Guantity (g/dm^2)	Germination (%)	MGT	Root Length (mm)	Shoot Length (mm)	SVI
E. oryzoides	0.00	100.0 ± 0.0	5.0 ± 0.0 a	92.7 ± 3.6 a	36.5 ± 1.5	12925 ± 371 a
	0.4	92.5 ± 9.6	5.3 ± 0.1 ab	43.1 ± 24.5 b	37.6 ± 3.7	7444 ± 2530 b
	0.8	95.0 ± 5.0	5.4± 0.3 b	38.4 ± 11.4 b	35.3 ± 2.9	6973 ± 1180 b
	F	1.964	7.226	20.427	0.780	23.126
	p-value	0.186	0.011 *	0.000 *	0.482	0.000 *
O. sativa	0.00	90.0 ± 10.0	5.0 ± 0.1	64.7 ± 3.9	27.6 ± 1.9	8297 ± 804
	0.4	90.0 ± 7.1	5.2 ± 0.1	55.8 ± 19.6	23.8 ± 4.1	7214 ± 2381
	0.8	88.8 ± 4.5	5.1 ± 0.1	54.3 ± 11.9	21.4 ± 5.4	6632 ± 1338
	F	0.118	2.000	0.878	2.923	1.321
	p-value	0.890	0.178	0.441	0.092	0.303
Interaction species × treatment						
	F	0.762	3.384	6.715	1.588	5.573
	p-value	0.478	0.052	0.005 *	0.227	0.011 *

Values are mean ± standard deviation, asterisk and different letters indicate statistically significant differences at *p*-value ≤ 0.05 among treatments in each species. F-value and *p*-value of the ANOVA test. MGT, mean germination time; SVI, seedling vigor index.

No significant results were detected for *O. sativa* in relation to the measured indices. Similarly, stems were not able to affect germination percentage and shoot growth of *E. oryzoides*. On the other hand, at 0.4 and 0.8 g/dm^2, the treatment increased its MGT by 8% and decreased the root length up to 59% by significantly influencing SVI, reduced by 42% and 46%, respectively. In this case, the interaction "species × *L. multiflorum* stem powder treatment" was significant (*p*-values ≤ 0.05) only for root length and SVI.

In general, the results obtained for the *L. mutiflorum* stems showed a higher susceptibility of the weed than the crop in their responses to the increasing concentrations (Tables 1 and 2).

2.2. Inflorescence Effects

Similarly to the stems, *L. multiflorum* inflorescence extract affected the seed development of both studied species showing a greater inhibitory action on *E. oryzoides* compared to *O. sativa*, particularly on the three seedling growth parameters, namely SVI, root and shoot length (*p*-values < 0.05 for the interaction "species × *L. multiflorum* inflorescence extract treatment") (Table 3). Otherwise, there is no preferential effect by the extract in reducing the germination of the one of the two species.

Their germination percentage was remarkably lowered by 50% and 100% extract concentrations (*p*-values = 0.000). In the first case, the germinated seeds of *O. sativa* and *E. oryzoides* were 38% and 18%, respectively, while 100% extract concentration was able to completely inhibit them (0% germination). In addition, the 50% extract concentration decreased root length of both species by 83% and 92% than controls, as well as their shoot length (−33% and −70%) by significantly reducing the corresponding SVI values (−86% and −95%). Notably, the *E. oryzoides* shoot elongation was also affected by inflorescence 20% extract concentration (−26%).

Like the extract, also *L. multiflorum* powdered inflorescences placed in direct contact with *O. sativa* and *E. oryzoides* seeds significantly affected all measured indices, except for MGT (Table 4).

Table 3. Germination indices measured for *E. oryzoides* and *O. sativa* under the effect of different concentrations of *L. multiflorum* inflorescence extract.

Species	Inflorescence Extract Concentration (%)	Germination (%)	Mean Germination Time	Root Length (mm)	Shoot Length (mm)	Seedling Vigor Index
E. oryzoides	0	100.0 ± 0.0 a	5.0 ± 0.0	66.2 ± 14.5 a	34.0 ± 3.9 a	9008 ± 1764 a
	1	83.2 ± 7.5 a	5.6 ± 0.5	76.0 ± 1.0 a	33.3 ± 2.0 a	7733 ± 264 a
	10	90.2 ± 5.6 a	5.8 ± 0.4	68.0 ± 9.0 a	29.3 ± 2.5 ab	8405 ± 978 a
	20	94.8 ± 3.0 a	5.6 ± 0.5	62.0 ± 4.0 a	25.0 ± 0.6 b	9255 ± 772 a
	50	18.0 ± 21.7 b	5.3 ± 0.6	4.3 ± 0.6 b	10.3 ± 2.1 c	407 ± 276 b
	100	0.0 ± 0.0 c	n.d.	n.d.	n.d.	n.d.
	F	100.627	2.179	27.971	39.963	32.699
	p-value	0.000 *	0.113	0.000 *	0.000 *	0.000 *
O. sativa	0	96.8 ± 4.6 a	5.0 ± 0.0	43.0± 13.5 a	19.8 ± 2.6 a	6072 ± 1525 a
	1	96.8 ± 5.6 a	4.6 ± 0.5	44.3 ± 1.5 a	17.7 ± 0.6 a	5794 ± 508 a
	10	95.0 ± 5.4 a	4.6 ± 0.5	40.3 ± 3.5 a	18.3 ± 1.5 a	4825 ± 222 a
	20	88.4 ± 2.6 a	5.0 ± 0.7	48.7 ± 1.5 a	15.2 ± 4.1 ab	5806 ± 327 a
	50	37.0 ± 20.1 b	5.2 ± 0.4	7.2 ± 3.4 b	13.3 ± 2.5 b	825 ± 554 b
	100	0.0 ± 0.0 c	n.d.	n.d.	n.d.	n.d.
	F	101.674	1.385	21.825	3.155	27.790
	p-value	0.000 *	0.275	0.000 *	0.048*	0.000 *
	Interaction species × treatment					
	F	1.39	2.764	4.45	17.641	4.656
	p-value	0.265	0.051	0.007 *	0.000 *	0.006 *

Values are mean ± standard deviation, asterisk and different letters indicate statistically significant differences at p-value ≤ 0.05 among treatments in each species. F-value and p-value of the ANOVA test. MGT, mean germination time; SVI, seedling vigor index.

Table 4. Germination indices measured for *E. oryzoides* and *O. sativa* under the effect of different quantity of *L. multiflorum* powdered inflorescences.

	Powdered Inflorescence Quantity (g/dm^2)	Germination (%)	MGT	Root Length (mm)	Shoot Length (mm)	SVI
E. oryzoides	0.00	100.0 ± 0.0 a	5.0 ± 0.1	90.6 ± 5.9 a	36.2 ± 1.7 a	12688 ± 647 a
	0.4	56.4 ± 35.1 b	5.2 ± 0.2	9.8 ± 9.9 b	28.8 ± 3.7 b	2649 ± 627 b
	0.8	22.0 ± 31.9 b	5.6 ± 0.8	2.9 ± 0.8 b	14.9 ± 5.0 c	920 ± 57 c
	F	10.165	2.121	175.721	33.891	436.013
	p-value	0.003 *	0.182	0.000 *	0.000 *	0.000 *
O. sativa	0.00	92.0 ± 8.4 a	5.0 ± 0.0	63.2 ± 5.1 a	25.4 ± 1.3 a	8122 ± 355 a
	0.4	72.5 ± 10.9 b	5.1 ± 0.1	12.6 ± 8.3 b	18.8 ± 6.4 b	2401 ± 1489 b
	0.8	30.0 ± 12.2 c	5.0 ± 0.0	3.5 ± 1.1 c	11.3 ± 1.4 c	466 ± 223 c
	F	44.506	2.889	160.545	16.963	99.309
	p-value	0.000 *	0.095	0.000 *	0.000 *	0.000 *
	Interaction species × treatment					
	F	2.292	3.357	15.724	2.014	21.465
	p-value	0.127	0.055	0.000 *	0.160	0.000 *

Values are mean ± standard deviation, asterisk and different letters indicate statistically significant differences at p-value ≤ 0.05 among treatments in each species. F-value and p-value of the ANOVA test. MGT, mean germination time; SVI, seedling vigor index.

The germination percentage decreased by 21%–67% and 44%–78%, respectively; the root length by 80%–94% and 89%–97%, shoot length by 26%–56% and 21%–59%, SVI by 70%–94% and 79%–93%, due to both used quantities (0.4 and 0.8 g/dm^2). The species showed a similar response to the treatments both as regards the germination percentage and the shoot length (p-values > 0.05). Accordingly, the interaction "species × *L. multiflorum* inflorescence powder treatment" was significant only for root length and SVI (*p*-values = 0.000) confirming the tendency towards greater susceptibility of *E. oryzoides* shown by the previous results.

2.3. Root Effects

Unlike stems and inflorescences, *L. multiflorum* root extract was not able to affect, at any used concentration, both MGT and germination percentage in the studied species. Furthermore, only 50% and 100% extract concentrations showed cases of significant impact on other considered indices (Table 5).

Table 5. Germination indices measured for *E. oryzoides* and *O. sativa* under the effect of different concentrations of *L. multiflorum* root extract.

Species	Root Extract Concentration (%)	Germination (%)	MGT	Root Length (mm)	Shoot Length (mm)	SVI
E. oryzoides	0	100.0 ± 0.0	5.0 ± 0.0	66.2 ± 14.5 a	34.0 ± 3.9 a	9008 ± 1764 a
	1	93.2 ± 7.5	5.6 ± 0.5	68.3 ± 3.5 a	32.0 ± 1.0 a	8740 ± 120 a
	10	91.6 ± 2.6	5.6 ± 0.5	63.7 ± 1.5 a	32.3 ± 0.6 a	8787 ± 123 a
	20	93.2 ± 4.6	5.4 ± 0.5	67.0 ± 7.0 a	32.0 ± 2.0 a	9557 ± 537 a
	50	98.0 ± 4.5	5.2 ± 0.4	46.8 ± 4.0 b	32.2 ± 4.1 a	7734 ± 1040 a
	100	92.0 ± 8.4	5.4 ± 0.5	21.8 ± 2.9 c	24.8 ± 2.7 b	4321 ± 820 b
	F	1.870	1.171	22.461	5.315	14.805
	p-value	0.140	0.352	0.000 *	0.004 *	0.000 *
O. sativa	0	96.8 ± 4.6	5.0 ± 0.0	43.0± 13.5 a	19.8 ± 2.6 a	6072 ± 1525 a
	1	100.0 ± 0.0	5.0 ± 0.0	43.0 ± 4.0 a	20.7 ± 3.5 ab	6323 ± 752 a
	10	95.0 ± 5.4	4.6 ± 0.5	48.7 ± 1.5 a	25.3 ± 1.5 a	6920 ± 724 a
	20	94.8 ± 3.0	4.8 ± 0.4	47.6 ± 3.5 a	25.0 ± 2.0 a	6760 ± 579 a
	50	96.2 ± 3.3	5.0 ± 0.0	57.6 ± 6.0 a	26.4 ± 4.5 a	7784 ± 1184 a
	100	92.2 ± 6.1	5.0 ± 0.0	23.0 ± 6.5 b	14.4 ± 4.4 b	3304 ± 696 b
	F	1.867	1.680	10.479	7.595	10.370
	p-value	0.138	0.178	0.000 *	0.001 *	0.000 *
Interaction species × treatment						
	F	2.371	1.543	6.227	2.357	2.761
	p-value	0.59	0.201	0.000 *	0.06	0.033 *

Values are mean ± standard deviation, asterisk and different letters indicate statistically significant differences at *p*-value ≤ 0.05 among treatments in each species. F-value and *p*-value of the ANOVA test. MGT, mean germination time; SVI, seedling vigor index.

At 100% extract concentration, a reduction by 27% was observed in the *O. sativa* and *E. oryzoides* shoot length (*p*-values < 0.05) and by about 50% for their SVI values (*p*-values = 0.000). Roots decreased by 47% and 61% (*p*-values = 0.000), respectively.

Lastly, the significant interaction "species × *L. multiflorum* root extract treatment" with respect to root length and SVI (*p*-values < 0.05), thanks also to the effect of the 50% extract concentration on the roots of *E. oryzoides* (−17%), showed a greater inhibition of the growth of weed seedlings compared to that of rice.

The results of *L. multiflorum* root powder bioassay supported previous data on *E. oryzoides* showing that both used quantities (0.4 and 0.8 g/dm^2) significantly influenced only its root elongation (decrease between 36% and 38% compared to the control) and SVI (decrease between 29% and 30%). Contrastingly, the powdered roots showed no effect against *O. sativa*, for which all the values of the measured indices were comparable to those of controls (Table 6). On the basis of these results, the interaction "species × *L. multiflorum* root powder treatment" was significant, showing a greater reduction in *E. oryzoides* root length and SVI.

Table 6. Germination indices measured for *E. oryzoides* and *O. sativa* under the effect of different quantity of *L. multiflorum* powdered roots.

Species	Powdered Root Quantity (g/dm^2)	Germination (%)	MGT	Root Length (mm)	Shoot Length (mm)	SVI
E. oryzoides	0.00	100.0 ± 0.0	5.0 ± 0.1	90.6 ± 5.9 a	36.2 ± 1.7	12688 ± 647 a
	0.4	94.0 ± 5.5	5.0 ± 0.1	57.9 ± 9.7 b	37.3 ± 1.4	8984 ± 1389 b
	0.8	94.0 ± 5.5	5.1 ± 0.2	56.2 ± 21.9 b	39.2 ± 3.9	8872 ± 1965 b
	F	3.000	1.471	9.275	1.704	11.394
	p-value	0.088	0.268	0.004 *	0.223	0.002 *
O. sativa	0.00	92.5 ± 8.3	5.0 ± 0.0	63.2 ± 5.1	25.4 ± 1.3	8166 ± 346
	0.4	92.0 ± 8.4	5.0 ± 0.1	62.7 ± 12.6	31.8 ± 4.5	8639 ± 1427
	0.8	90.0 ± 7.1	5.1 ± 0.1	52.7 ± 8.4	29.5 ± 5.3	7478 ± 1697
	F	0.139	1.600	2.063	3.205	1.015
	p-value	0.872	0.242	0.170	0.077	0.391
	Interaction species × treatment					
	F	0.467	0.286	4.843	1.724	6300
	p-value	0.632	0.754	0.017 *	0.200	0.006 *

Values are mean ± standard deviation, asterisk and different letters indicate statistically significant differences at *p*-value ≤ 0.05 among treatments in each species. F-value and *p*-value of the ANOVA test. MGT, mean germination time; SVI, seedling vigor index.

2.4. Seed Effects

The phytotoxic activity of *L. multiflorum* seeds was also assessed. Their aqueous extract impacted similarly on both target species that achieved growth values comparable to those of their controls for all considered indices (*p*-values > 0.05) (Table 7). The germination percentage of treated *E. oryzoides* and *O. sativa* was greater than 90%. MGT was the same as for untreated seeds while the root and shoot development showed insignificant differences as well as SVI values (*p*-values > 0.05).

Table 7. Germination indices measured for *E. oryzoides* and *O. sativa* under the effect of *L. multiflorum* seed extract.

Species	Seed Extract	Germination (%)	MGT	Root Length (mm)	Shoot Length (mm)	SVI
E. oryzoides	0%	96.7 ± 5.2	5.7 ± 0.4	42.0 ± 6.5	23.5 ± 3.0	6323 ± 785
	100%	91.7 ± 20.4	5.7 ± 0.1	50.8 ± 13.7	24.5 ± 7.2	6703 ± 1866
	F	0.338	0.037	2.034	0.086	0.212
	p-value	0.574	0.850	0.184	0.775	0.655
O. sativa	0%	90.0 ± 25.3	4.7 ± 0.1	51.4 ± 5.1	23.3 ± 5.1	5432 ± 2640
	100%	98.0 ± 12.1	4.7 ± 0.1	50.1 ± 3.6	21.8 ± 2.7	6040 ± 1308
	F	1.356	0.448	0.271	0.425	0.256
	p-value	0.271	0.519	0.614	0.529	0.624
	Interaction species × treatment					
	F	1.640	0.146	2.301	0.387	0.024
	p-value	0.215	0.706	0.145	0.541	0.878

Values are mean ± standard deviation. F-value and *p*-value of the ANOVA test. MGT, mean germination time; SVI, seedling vigor index.

2.5. Polyphenol Content in *L. multiflorum* Extracts

Figure 1 shows the polyphenol content in the aqueous extracts of the different investigated *L. multiflorum* parts measured using the Folin-Ciocalteau reagent.

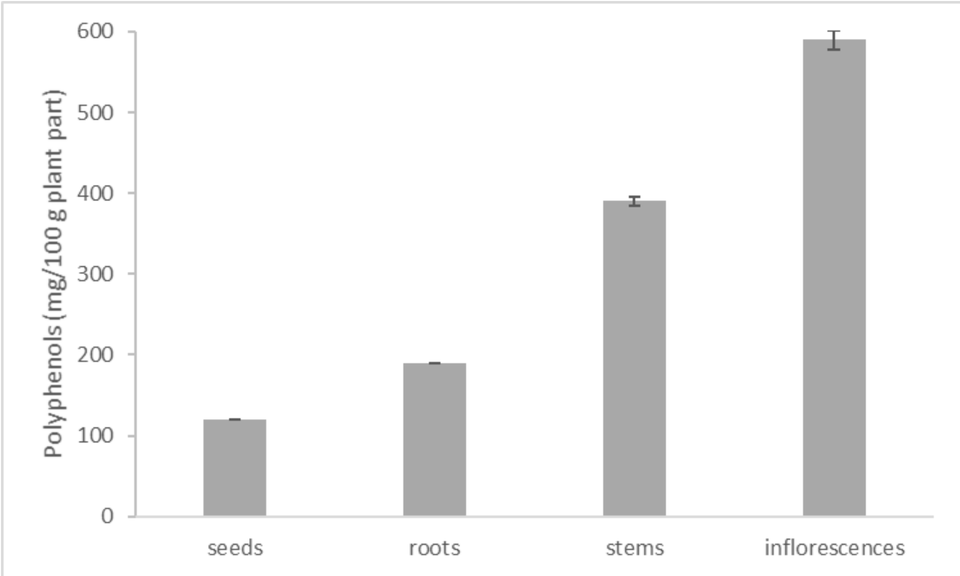

Figure 1. Total polyphenols detected in the aqueous extracts of the various *L. multiflorum* organs.

The highest content, equal to 590 mg GAE/100 g plant part, was identified in the inflorescences extract. Gradually lower quantities were found in stems (390 mg GAE/ 100 g), roots (190 mg GAE/ 100 g) and seeds (120 mg GAE/100 g), in accordance with the decreasing phytotoxic activity recorded for the various *L. multiflorum* parts against *E. oryzoides* and *O. sativa*.

3. Discussion

Different studies reported the allelopathic activity of some *Lolium* species including *L. multiflorum* [25–28]. Usually, it is treated more as a weed capable of undermining the crop rather than as a crop cultivated with a function in the weed control and, therefore, in the crop protection. For example, Lehoczky and co-workers [26] described the inhibitory effects of aqueous extract obtained from *L. multiflorum* shoots on some of the main grown crops such as *Hordeum vulgare* L., *Triticum aestivum* L. and *Zea mays* L. However, other authors investigated the impact of decaying residues from *L. multiforum* used as a cover crop on *O. sativa* seedling development obtaining opposite results due to both inhibitory and stimulating effects [29–31]. To the best of our knowledge, very few data refer to the effectiveness of *L. multiflorum* against the weed growth [32]. Moreover, the relationship between *L. multiflorum* and *E. oryzoides* was never investigated.

In this context, our results are particularly interesting and can be at the basis of the weed management strategies adopted by organic farmers who cultivated *L. multiflorum* before rice.

L. multiflorum showed a preferential action with impacts significantly different and more severe on *E. oryzoides* rather than on *O. sativa*. Both *L. multiflorum* treatments, i.e., aqueous extracts and powder, obtained from all the investigated organs—inflorescences, stems and roots—showed a significant inhibitory effect on the weed. In particular, the stem and inflorescence aqueous extracts and the inflorescence powder significantly affected both seed germination and seedling growth, while the root aqueous extract and the stem and root powder reduced the root length. The root development of *E. oryzoides* showed always a greater reduction than those of *O. sativa*. In addition, species-specific phytotoxic effects were evident for the inflorescence and stem extracts, also regarding the shoot development and seed germination.

Additionally, *O. sativa* suffered the inhibitory effect of the aqueous extracts from *L. multiflorum*. In particular, inflorescence and stem extracts were able to reduce both the seed germination and the seedling growth (i.e., root and shoot elongation), while the root extracts affected only the seedling growth. On the other hand, the powder treatments showed minor activity and only those obtained from inflorescence had significant effect, inhibiting the seed germination and the seedling growth.

Therefore, the data showed the existence of a phytotoxic activity by *L. multiflorum*, instead of its stimulating effects on rice, and, in general, a more marked action of the inflorescence, followed by stems and roots.

Finally, the seed aqueous extract was unable to affect *E. oryzoides* neither *O. sativa*. In both bioassays, all their growth parameters reached high values, similar to those of controls. The ineffectiveness of *L. multiflorum* seeds in influencing the development of other seeds could be attributed to the fact that the phytotoxic substances present in the cover crop are synthesized in a subsequent growth stage of the plant.

The preferential impact of *L. multiflorum* on the root development confirmed previous data documenting that the phytotoxic effect most observed in vegetative structures occurs on the root system [20]. Nevertheless, some studies on the relationships between species showed the different impact of the aqueous biomass extract on the measured variables. For example, Hoffman et al. [33] reported significant inhibition of root and shoot growth, without effect on germination, while Turk et al. [34] documented the decrease of germination and no reduction of the hypocotyl as well as Han et al. [35] recorded the inhibition of germination and root development but no effect on the shoots. On the other hand, the activity of phytotoxic compounds and their effects such as reduction in seed germination and seedling growth are caused by a variety of specific interactions and cannot been explained by just a single mode of action [28].

Lastly, polyphenols are a heterogeneous group of substances produced by the secondary metabolism of plants, where, in relation to chemical diversity, they play different roles. They can be simple low molecular weight compounds or complex structures conjugated with sugar moieties useful to plants for their structure, pigmentation, pollination, defense from predators and pathogens. Furthermore, their action as allelochemicals is known and investigated [36–39]. The different phytotoxicity of the investigated *L. multiflorum* organs could be partially related to the decreasing polyphenol values detected starting from the inflorescences.

Some allelopathic compounds were previously isolated from the aqueous leachates of decaying *L. multiflorum* residues. In particular, benzenepropanoic acid has proven to be effective in inhibiting the root and shoot growth of rice seedlings [29]. Other compounds such as caffeic acid, *p*-coumaric acid, ferulic acid and hydrocinamic acid were identified in the water fraction obtained from the fermentation of *L. multiflorum* shoots and roots. These phenolic acids seem to be responsible for the ability of the extract to reduce the shoot and root elongation in different rice cultivars [31]. Furthermore, the same type of extract was also able to affect the growth of two wheat cultivars [32].

In conclusion, the data obtained from our in vitro tests substantially confirmed the farmer empirical observations regarding the use of *L. multiflorum* as a cover crop, namely the corresponding reduction of *E. oryzoides* incidence, and explained the *O. sativa* poor density observed in certain fields under the same practice. In their opinion, *L. multiflorum* appears to negatively affect both the weed and rice development in the early growth stages, but *O. sativa* is less influenced than *E. oryzoides*, and on this thin difference it is possible to play for giving the crop a competitive advantage over the weed.

The farmers' empirical knowledge comes from their direct long-time experiences in managing complex agro-ecosystems or are drawn from the rural tradition, thus including and safeguarding a stock of precious knowledge often fragmented or lost. It could be not a coincidence that in the past century, when the local farms combined the rice production with livestock, the cultivation of forage species such as *L. multiflorum* in rotation with rice was a common practice. Hence, the study results validate the usefulness of the farmers' contribution in participatory research as a valuable guide for scientific inquiry and as a support for innovations in sustainable agriculture.

Moreover, *L. multiflorum* could be considered the starting point to formulate new plant-based and eco-friendly herbicides, functional to reduce the use of more dangerous synthetic compounds and the consequent environmental pressure due to agronomic practices in the rice area.

4. Materials and Methods

4.1. Plant Material

Seeds of *O. sativa* (cv. Rosa Marchetti), *E. oryzoides* and *L. multiflorum* were obtained from 'Terre di Lomellina' organic farm located in the northern Italy (GPS coordinates: 45°10′28.329′′N 8°35′44.198′′E). They were stored at 4 °C until use after surface sterilization with 1% sodium hypochlorite by shaking for 7 min and repeatedly rinsing with distilled water.

In the same farm, fresh plants of *L. multiflorum* were also collected. Their inflorescences, stems and roots were separately air-dried at room temperature (25 °C) in the shade and preserved in paper bags until extraction.

4.2. Aqueous Extract Bioassay

The aqueous extract of each powdered part—inflorescences, stems and roots—of *L. multiflorum* was prepared mixing a suitable amount with distilled water (1:10, *w*/*v*) and shaking it at room temperature for 24 h. Afterwards, the mixture was filtered through gauzes to remove residues and centrifuged at 4500 rpm for 30 min. The obtained extracts were used as such (100%) and diluted with distilled water to give final concentrations of 1%, 10%, 20% and 50%.

Otherwise, in order to simulate the leaching from seeds, 60 unsterilized seeds were placed in 30 mL of distilled water on an orbital shaker, at room temperature, for 24 h. Subsequently, the obtained extract was filtered before use.

Ten sterilized seeds of *E. oryzoides* and *O. sativa* were sown into each Petri dish (90 mm diameter) containing 2 filter papers and 4 mL of each extract or its dilution were added. The same volume of distilled water was used as a control (0%). Petri dishes prepared in a vertical laminar flow hood and sealed with parafilm were kept in a growth chamber (25 °C/16 h light and 18 °C/8 h dark) for seven days.

Concerning the inflorescence, stem and root extracts, five Petri dishes were realized for each combination of "species × *L. multiflorum* treatment", according with the following randomized block design: two species (*E. oryzoides* and *O. sativa*) × six levels of concentration (100%, 50%, 20%, 10%, 1%, 0%) × three *L. multiflorum* organs (inflorescences, stems and roots) × five replicates. A similar experimental design with five repetitions was followed for the seed extract, considering only two levels of concentration (100% and 0%) and one *L. multiflorum* organ (seed).

4.3. Plant Part Powder Bioassay

Different quantities (0.4 and 0.8 g/dm^2) of each powdered part—inflorescences, stems and roots—of *L. multiflorum* were spread on two filter papers in Petri dishes (90 mm diameter). Afterwards, ten sterilized seeds of *E. oryzoides* and *O. sativa*, respectively, were placed and soaked with 5 mL of distilled water. The same volume of distilled water was used in the control samples (0 g/dm^2 of powder). Petri dishes prepared in a vertical laminar flow hood and sealed with parafilm were kept in a growth chamber (25 °C/16 h light and 18 °C/8 h dark) for seven days. Five Petri dishes were realized for each combination of "species × *L. multiflorum* treatment" according with the following randomized block design: two species (*E. oryzoides* and *O. sativa*) × three levels of quantity (0.5 g, 0.25 g, 0 g) × three *L. multiflorum* organs (inflorescences, stems and roots) × five replicates.

4.4. Seedling Growth Parameter and Germination Indices

The number of germinated seeds in each Petri dish was recorded daily. At the seventh day, the length of their radicles and shoots was measured on graph paper under a stereomicroscope. The collected data were used to calculate the germination percentage, SVI [40] and MGT [41], respectively, by the following equations:

$$\text{Germination Percentage} = \frac{\textit{Germinated Seed Number}}{\textit{Seed Total Number}} \times 100 \quad (1)$$

$$\text{SVI} = (\textit{Mean Root Length} + \textit{Mean Shoot Length}) \times \textit{Germination Percentage} \quad (2)$$

$$\text{MGT} = \frac{\sum D \times \textit{Germinated Seed Number}}{\sum \textit{Germinated Seed Number}}, \quad (3)$$

where D is the number of days from the beginning of germination.

4.5. Determination of Polyphenolic Content

The total polyphenolic content of the aqueous extracts was determined colorimetrically by the Folin-Ciocalteau method described by Scalbert et al. [42] with slight modifications. Briefly, 0.5 mL of each extract was added to 2.5 mL of 10% Folin-Ciocalteau reagent, previously diluted with distilled water. After 3 min, 2 mL of 7.5% sodium carbonate solution was added. The mixture was incubated in the dark for 1 h at room temperature and its absorbance was measured at 765 nm using a UV-vis spectrophotometer (Jenway 7205). A calibration curve was prepared with gallic acid standard solution at various concentrations (10 to 100 mg/L). The results were expressed as mg gallic acid equivalent (GAE)/100 g dry plant part. All the measurements were taken in triplicate and the mean values were calculated.

4.6. Statistical Analysis

The data were analyzed, with the support of IBM SPSS software, through the analysis of variance carried out separately for each bioassay (i.e., extract and powder bioassays) and *L. multiflorum* organs (i.e., inflorescences, stems, roots, seeds). The germination indices (i.e., germination percentage, SVI, MGT, root length, shoot length) measured for the two species (i.e., *E. oryzicola* and *O. sativa*) under different treatments were taken into account as dependent variables.

The one-way ANOVA and the Turkey's-b post hoc test were performed in order to establish the significant effect (at $\alpha \leq 0.05$) of the treatments with *L. multiflorum* (i.e., the different levels of concentration or quantity in extract and powder bioassay, respectively), on the species, and describe the homogenous subsets.

Moreover, the two-way ANOVA was performed, considering as factors the treatments with *L. multiflorum* and the species, in order to highlight the significant interaction (at $\alpha \leq 0.05$) between "species × *L. multiflorum* treatments", and then highlighting the species-specific effects of the treatments and the different behaviors or susceptibility between the rice crop and the weed.

Author Contributions: Co-first authors, S.V. and F.O; co-last authors, S.B. and M.I. Conceptualization, S.B., M.I. and S.V.; methodology, F.O., M.I., V.V. and S.V.; validation, M.I. and S.V.; formal analysis, F.O. and V.V.; investigation, V.V. and S.V.; resources, S.B. and M.I.; data curation, F.O. and S.V.; writing—original draft preparation, F.O. and S.V.; writing—review and editing, F.O., M.I. and S.V.; visualization, F.O., V.V. and S.V.; supervision, M.I. and S.V.; project administration, M.I. and S.V.; funding acquisition, S.B. and M.I. All authors have read and agreed to the published version of the manuscript.

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CORRECTION

Correction to: Different phytotoxic effect of *Lolium multiflorum* Lam. leaves against *Echinochloa oryzoides* (Ard.) Fritsch and *Oryza sativa* L.

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In the title, it should be Oryza instead of Oriza.

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Francesca Orlando was co-first author and Stefano Bocchi was co-last author.

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RESEARCH ARTICLE

Different phytotoxic effect of *Lolium multiflorum* Lam. leaves against *Echinochloa oryzoides* (Ard.) Fritsch and *Oriza sativa* L.

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Abstract

Rice cultivation, particularly prone to weed issues, requires practices able to effectively control them, however reducing the use of herbicides, responsible for damage to human health and ecosystem sustainability. Alternative strategies for weed management can be based on plant-plant interaction phenomena. In this context, a group of organic farmers has developed a pragmatic approach for weed containment using *Lolium multiflorum* Lam. as a cover crop before rice. The present study aimed to confirm the farmer field observations reporting a preferential inhibitory effect of *L. multiflorum* on *Echinochloa oryzoides* (Ard.) Fritsch, one of the most yield-damaging rice weed, compared with *Oryza sativa* L. The study showed that *L. multiflorum* was able to significantly reduce the seed germination of *E. oryzoides*. It was found to be more susceptible than *O. sativa* both to the effect of the aqueous extract and powder of *L. multiflorum* leaves (23–79% vs. 3–57% and 26–100% vs. 23–31%, respectively). In addition, the leaf extract was able to affect *E. oryzoides* growth starting from 20% concentration both in relation to the root and shoot length while *O. sativa* exhibited differences compared with the control only under the influence of extract 50%. The *L. multiflorum* leaf characterization by NMR and UPLC-HR-MS analyses led to the identification of 35 compounds including several polyphenols, glycosyl flavonoids and glycosyl terpenoids, as well as different amino acids and organic acids. Some of them (e.g. protocatechuic and gallic acids) are already known as allelochemicals confirming that *L. multiflorum* is a source of plant growth inhibitors.

Keywords Allelopathy · Cover crop · Early watergrass · Italian ryegrass · Organic rice · Weed biocontrol

Introduction

Weed control strategies play a key role in the success of agricultural production. This is even more true for rice, a crop particularly prone to weed issues. The incidence of weeds is the main constraint for rice production and the main cause of the yield gap between conventional and organic farming (Delmotte et al. 2001; Shennan et al. 2017; Hazra et al. 2018). On the other hand, the intensive use of herbicides in high-input cropping systems is becoming increasingly problematic in terms of environmental pollution. Their low biodegradability and high persistence pose considerable hazards for soil and water quality, and, to a certain extent, for human health (Kim et al. 2017). Several European countries report levels for one or more pesticides in groundwater that exceed quality standards. In some areas of northern Italy, between Piedmont and Lombardy regions, where 94% of national rice production is concentrated, the highest degradation of groundwater and surface water quality due to pesticide and herbicide

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contamination was found (Ispra 2018). For all these reasons, the European authorities have prohibited the use of active substances potentially harmful to human health or the environment (e.g. Directive 79/117/EE; Directive 91/414/CEE).

In addition to the restrictions on the use of certain chemicals belonging to traditional phytosanitary plans, there are also problems related to the progressive ineffectiveness of some products in the control of weed populations. The wide and continuous use of herbicides with a limited diversification of the action mechanisms in mono-cropping systems has caused resistance phenomena worldwide. Among rice weeds, some *Echinochloa* species such as *E. colona* (L.) Link, *E. crus-galli* L., *E. oryzoides* (Ard.) Fritsch have evolved herbicide resistance in many countries by reducing farm productivity (Fischer et al. 2000; Talbert and Burgos 2007; Malik et al. 2010; Wright et al. 2018). In particular, *E. oryzoides* was found resistant to ALS-inhibitors, ACCase-inhibitors and lipid inhibitors (Fischer et al. 2000; Altop et al. 2014). *E. oryzoides*, known as early watergrass, is also particularly fearsome for the rice production because it emerges before the other *Echinochloa* species, thus reaching greater competitiveness. This results in a lower rice plant density and severe yield losses.

Against this background, new weed management strategies, able to delay or reduce their germination and growth, are necessary (Gibson et al. 2002; Awan et al. 2015). The development of eco-friendly herbicides or agroecological practices effective in reducing the *Echinochloa* species incidence is a key aspect to enhance the sustainability of the rice cultivation. Plant-plant interference, intended as a negative effect of one plant on another, is a widely investigated aspect to find a useful alternative tool (Kadioglu and Yanar 2004; Jose et al. 2016; Lim et al. 2017). In the agroecosystem integrated management, the introduction into crop rotations of allelopathic crops affecting the weeds' growth is a promising agroecological practice (Wezel et al. 2014). In this regard, some interesting results emerged from the on-farm research carried out by a group of researchers and some organic rice farmers in North Italy (Orlando et al. 2020). The participatory research identified a promising management strategy for weed control within the organic rice cropping system. After the cultivation of a winter herbage as a cover crop, the rice no-till sowing is carried out directly on the standing cover crop. It is not incorporated into the soil as green manure but shredded and used as green mulching, briefly irrigated before the rice paddy flooding, postponed for about a month. The farmer experience has highlighted that when *Lolium multiflorum* Lam. is chosen as a cover crop, a considerable reduction of *E. oryzoides* incidence is detected. These observations suggested the existence of a species-specific mechanism of interaction between the two species, beyond the well-known effect of competitiveness for light, nutrients and space (Teasdale 1996; Bastiaans et al. 2008). Based on their empirical knowledge, farmers have speculated that the presence of the *L. multiflorum* green mulching affects both rice and weed growth. Nevertheless, the weed is more susceptible, and this difference can be useful to obtain a competitive advantage in favour of rice. To validate this hypothesis, the possibility of successfully managing a plot- or field-scale experiment was evaluated untenable. The organic rice field is, in fact, a complex system where many variability sources interact in the short and long term, contributing to determine the weeds dynamics, with resulting high inter- and intra-field and inter- and intra-season variability (Stoop et al. 2009; Orlando et al. 2020).

Accordingly, the present study was aimed to verify in vitro the phytotoxic activity of *L. multiflorum* leaves against *E. oryzoides* integrating our previous results (Vitalini et al. 2020). Likewise, the possible effects on *O. sativa* were also evaluated and different approaches simulating the release of phytotoxic compounds from the producing plant towards the target plant were considered.

Materials and methods

Plant material

Seeds of *E. oryzoides* were collected during 2018 from an organic rice field of the 'Terre di Lomellina" farm, located in the province of Pavia (North-West Italy). The same farm provided the seeds of *O. sativa* L. (cv. Rosa Marchetti) and *L. multiflorum*. All seeds were stored at 4 °C. Before use, their surface was sterilized with 1% sodium hypochlorite by shaking for 10 min, then repeatedly rinsed with distilled water.

Furthermore, also *L. multiflorum* leaves were harvested in the rice fields of the "Terre di Lomellina" farm, air-dried at shade and room temperature (25 °C), then kept in paper bags until extraction. A voucher specimen (No. LMTL210) was deposited at the Department of Agricultural and Environmental Sciences, Milan State University (Milan, Italy), after its identification according to Flora d'Italia (Pignatti 1982).

Aqueous extract bioassay

To reproduce the field conditions, *L. multiflorum* aqueous extract was prepared as previously described (Vitalini et al. 2020). Powdered leaves were mixed with distilled water (1:10, w/v), then shaken at room temperature. After 24 h, the mixture was filtered through gauzes to remove residues and centrifuged at 2300 g for 30 min. The obtained extract was used as such (100%) as well as diluted with distilled water to give final concentrations of 1%, 10%, 20% and 50%. The seeds of *E. oryzoides* and *O. sativa* were sown in Petri dishes (9 cm) on filter paper. Ten sterilized seeds of each species were placed on two filter papers and soaked with 5 mL of each dilution. The same volume of distilled water was used as control (0% concentration). All Petri dishes were prepared in a vertical laminar flow hood by using sterile materials and sealed with parafilm before incubation in a growth chamber at 25 °C/16 h light and 18 °C/8 h dark cycle for 7 days. Five petri dishes were realized for each combination of "species × *L. multiflorum* treatment" by setting up the experimental design as follows: *E. oryzoides* or *O. sativa* seeds × 6 concentration levels of *L. multiflorum* extract (including distilled water as control) × 5 replicates.

Leaf powder bioassay

The leaf powder bioassay was carried out according to Vitalini et al. (2020) with some modifications. Different quantities (0.25 g, 0.36 g, 0.5 g) of *L. multiflorum* powdered leaves were spread on two filter papers in Petri dishes (9 cm). Then, ten sterilized seeds of *E. oryzoides* or *O. sativa*, respectively, were placed and 5 ml of distilled water was added. The same volume of distilled water was used in the control samples (0 g of leaf powder).

All Petri dishes were prepared in a vertical laminar flow hood by using sterile materials and sealed with parafilm before incubation in a growth chamber at 25 °C/16 h light and 18 °C/8 h dark cycle for 7 days. Five petri dishes were realized for each combination of "species × *L. multiflorum* treatment" by setting up the experimental design as follows: *E. oryzoides* or *O. sativa* seeds × 6 concentration levels of *L. multiflorum* extract (including distilled water as control) × 5 replicates.

Seed germination measurements

The seed germination was recorded daily. On the seventh day, root and shoot length (mm) of each germinated seed was measured on graph paper under a stereomicroscope and the average value per Petri dish was calculated. The obtained data were used to compute the following germination indices:

$$\text{Germination percentage} = \frac{Germinated\ seed\ number}{Seed\ total\ number} \times 100 \quad (1)$$

$$\text{SVI} = (Mean\ Root\ length + Mean\ Shoot\ length) \times Germination\ percentage \quad (2)$$

$$\text{MGT} = \frac{\sum D \times Germinated\ seed\ number}{\sum Germinated\ seed\ number} \quad (3)$$

where SVI is the Seedling Vigour Index (Eq. 2; Abdul-Baki and Anderson 1973) and MGT is the mean germination time (Eq. 3; Ellis and Roberts 1981) calculated taking into account D that is the number of days from the beginning of germination, plus the number of seeds germinated on day D.

Ultra-performance liquid chromatography/electrospray ionization-high resolution mass spectrometry (UPLC/ESI-HR-MS)

The UPLC/ESI-HR-MS analysis was carried by coupling an Acquity UPLC separation module (Waters, Milford, MA, USA) with in-line photodiode array (PDA) eλ detector (Waters) to a Q Exactive hybrid quadrupole-Orbitrap mass spectrometer and an HESI-II probe for electrospray ionization (Thermo Scientific, San Jose, CA, USA). The ion source and interface conditions were as follows: spray voltage + 3.5/− 3.5 kV, sheath gas flow 35, auxiliary gas flow 15, temperature 300 °C, and capillary temperature 350 °C. Positive mass calibration was performed with Pierce LTQ ESI Positive Ion Calibration Solution (Thermo Scientific Pierce, Rockford, IL, USA), containing caffeine, the tetrapeptide MRFA and Ultramark 1621. Negative mass calibration was performed with Pierce ESI Negative Ion Calibration Solution (Thermo Scientific Pierce), containing sodium dodecyl sulfate, sodium taurocholate and Ultramark 1621. Four μL of sample (20% diluted in water from crude extracts) were separated using a Waters Acquity BEH C18 column (150 × 2.1 mm, 1.7 μm, 130 Å) (Waters, Milford, MA, USA) kept at 40 °C, and using 0.1 mL 100 mL-1 of formic acid in H2O MilliQ-treated water (solvent A) and 0.1 mL 100 mL-1 formic acid in acetonitrile (solvent B). For the UPLC separation, a linear elution gradient was applied (isocratic 5% B for 5 min then 5% to 50% of solvent B in 20 min) at a flow rate of 0.2 mL min-1. The LC eluate was analysed by Full MS and data-dependent tandem MS analysis (dd-MS^2) of five of the most intense ions (Top 5). The resolution was set at 70,000 and 17,500 and the AGC targets were 1×10^6 and 1×10^5 for Full MS and dd-MS^2 scan types, respectively. The maximum ion injection times were 50 ms. The MS data were processed using Xcalibur software (Thermo Scientific) and Mnova MS plug-in (MestreNova 14.0.1, Mestrelab). Metabolites were determined according to their calculated exact mass and absorption spectra. Their structures were confirmed by high-resolution tandem MS (HR-MS/MS) by comparison with reported assignments in literature or databases.

NMR spectroscopy

Freeze-dried samples were suspended in D_2O at a final concentration of 10 mg/mL, sonicated (37 kHz, 20 min, Elmasonic P 30H, Elma Schmidbauer GmbH, Singen, Germany) and centrifuged (22,000 g, 5 min, 20 °C, ScanSpeed 1730R Labogene, Lynge, Sweden). 4,4-Dimethyl-4-silapentane-1-sulfonic acid (DSS, final concentration 0.5 mM) was added to the supernatant as internal reference for concentrations and chemical shift. The pH of each sample was verified with a microelectrode (Mettler Toledo, Columbus, OH, USA) and adjusted to 7.4 with NaOD or DCl. All pH values were corrected for the isotope effect. The acquisition temperature was 25 °C. All spectra were acquired on an Avance III 600 MHz NMR spectrometer (Bruker, Billerica, MA, USA) equipped with a QCI (^{1}H, ^{13}C, ^{15}N/^{31}P and ^{2}H) cryogenic probe. ^{1}H NMR spectra were recorded with *cpmgpr1d*, *noesygppr1d*, *ledbpgppr2s1d* pulse sequences (Bruker library) and 256 scans, a spectral width of 20 ppm, and a relaxation delay of 5 s. They were processed with 0.3-Hz line broadening, automatically phased and baseline corrected. Chemical shifts were internally calibrated to the DSS peak at 0.0 ppm. The ^{1}H,^{1}H-TOCSY (total correlation spectroscopy) spectra were acquired with 48 scans and 512 increments, a mixing time of 80 ms and relaxation delay of 2 s. ^{1}H,^{13}C-HSQC (heteronuclear single quantum coherence) spectra were acquired with 64 scans and 512 increments, relaxation delay 2 s. The NMR data were processed using MestreNova 14.1.0 software (Mestrelab Research, Santiago de Compostela, Spain). Compounds identification and assignment were done with the support of 2D NMR experiments, and comparison with reported assignments. For metabolite quantification, the simple mixture analysis tool (Cobas et al. 2011) integrated in MestreNova software package was exploited to set a semi-automatic protocol for the identification and quantification of metabolites, by creating specific metabolite libraries for the different analysed matrices. In this protocol, the global spectrum deconvolution algorithm was employed to deconvolute the overlapping regions and to perform the absolute quantification of metabolites with resonances in crowded spectral areas, too. When possible, the concentration was calculated looking at the mean value of the different signals assigned to the same metabolite.

Statistical analysis

The simple factorial experiment design with five replicates was followed. It contained the two target plant species (*E. oryzoides* and *O. sativa*) and the treatments performed with *L. multiflorum* extract (different concentration levels) or powder (different quantities) as fixed factors considered nominal and ordinal variables, respectively. In addition, the germination indices (germination percentage, SVI, MGT, root length and shoot length) were dependent variables. Analysis of variance was carried out separately for the aqueous extract bioassay and leaf powder bioassay. One-way ANOVA and the Tukey-B post hoc test evaluated the effects of *L. multiflorum* on *E. oryzoides* and *O. sativa* highlighting the most significant impacts. Two-way ANOVA analysed the interactions between the target species and the *L. multiflorum* extract or powder highlighting possible differences in the species response to the different treatments. The significance of F values was tested at p value ≤ 0.05. All statistical analyses were performed using the IBM SPSS.25.

Results

Leaf extract bioassay

Results of the aqueous extract bioassay are shown in Table 1. The interaction "species x *L. multiflorum* treatment" significantly affected the germination percentage, root length, shoot length and SVI (p values < 0.05) with differences between *E. oryzoides* and *O. sativa* in their responses to the increasing concentrations.

As for intra-species analysis, reductions in *E. oryzoides* germination were found at all five leaf extract concentrations (1% to 100%) compared with the distilled water used as a negative control (0%). In detail, 1%, 10% and 20% concentrations showed a similar impact, decreasing the seed germination by 26%, 23% and 30%, respectively. The 50% extract further reduced the number of germinated seed up to 42%. A strong effect was detected for 100% extract able to stop seed germination at 21% (i.e. seed germination decreased by 79%). Otherwise, the germination percentage of *O. sativa* remained unchanged compared with the control at all tested concentrations except at 100% leaf extract. Under its effect, the rice seeds showed a drastic reduction in germination by 57% less than the control.

Root length, shoot length and SVI (p values = 0.000) of *E. oryzoides* were significantly reduced by 20%, 50% and 100% extract concentrations. In particular, the root length decreased from 22% to 98%, shoot length from 30% to 85% and SVI from 55% to 97%, respectively. In the case of *O. sativa*, only 50% and 100% concentrations drastically impacted its roots (− 62% and − 90%, respectively) and the resulting SVI (− 59% and − 91%). Differently, all concentrations were unable to affect the shoot development (p value = 0.07). As for the MGT, both species showed a remarkable increase only under 100% extract effect (27% for *E. oryzoides* and 13% for *O. sativa*) without significant interaction (p value = 0.1).

Leaf powder bioassay

Concerning the plant powder bioassay, the obtained results are shown in Table 2. The interaction "species × *L. multiflorum* treatment" significantly affected the germination percentage (p value = 0.000) highlighting differences between *E. oryzoides* and *O. sativa* in their responses at 0.50 g of leaves, while the two species showed a similar trend with respect to the other germination indices (p value > 0.05).

The intra-species analysis showed that 0.50 g of *L. multiflorum* leaves completely suppressed *E. oryzoides* germination (0%), preventing the determination of the other

Table 1 Germination indices measured on filter paper for *E. oryzoides* and *O. sativa* under the effect of different concentrations of *L. multiflorum* leaf extract

Species	Leaf extract concentration (%)	Germination (%)	MGT	Root length (mm)	Shoot length (mm)	SVI
E. oryzoides	0	97.0 ± 4.5 a	5.5 ± 0.4 a	50.0 ± 11.9 b	25.2 ± 3.6 ab	7215 ± 1394 a
	1	71.7 ± 25.2 ab	5.6 ± 0.4 a	73.0 ± 4.5 a	28.6 ± 4.7 a	9070 ± 608 a
	10	75.0 ± 19.9 ab	5.5 ± 0.5 a	76.0 ± 5.9 a	33.2 ± 1.8 a	9783 ± 1174 a
	20	67.5 ± 5.0 ab	5.7 ± 0.5 a	39.1 ± 3.9 b	17.6 ± 2.9 b	3258 ± 1190 b
	50	56.7 ± 8.6 b	5.7 ± 0.2 a	10.3 ± 12.3 c	17.5 ± 3.7 b	1905 ± 1026 bc
	100	20.0 ± 23.1 c	7.0 ± 0.0 b	1.2 ± 1.5 c	3.7 ± 4.5 c	200 ± 240 c
	F	15.6	5.4	31.8	24.6	41.2
	p value	0.000*	0.002*	0.000*	0.000*	0.000*
O. sativa	0	98.1 ± 3.8 a	5.0 ± 0.2 a	39.4 ± 6.7 a	16.5 ± 3.8 a	5458 ± 999 a
	1	93.3 ± 5.4 a	4.8 ± 0.2 a	35.6 ± 16.0 a	12.4 ± 11.4 a	4384 ± 2693 ab
	10	93.3 ± 5.4 a	4.9 ± 0.3 a	38.8 ± 5.2 a	15.7 ± 4.0 a	4922 ± 1086 ab
	20	95.0 ± 6.4 a	5.0 ± 0.3 a	44.6 ± 7.9 a	18.9 ± 11.4 a	5666 ± 1442 a
	50	90.0 ± 0.0 a	5.2 ± 0.1 a	15.1 ± 11.7 b	9.8 ± 0.3 a	2242 ± 1050 bc
	100	42.5 ± 9.6 b	5.6 ± 0.1 a	3.8 ± 1.9 b	7.8 ± 0.5 a	500 ± 172 c
F		64.4	7.6	14.0	2.6	12.5
p value		0.000*	0.000*	0.000*	0.07	0.000*
Interaction species × treatment						
F		3.5	1.9	6.0	5.4	7.7
p value		0.009*	0.1	0.001*	0.001*	0.000*

Values are mean ± standard deviation, asterisk and different letters indicate statistically significant differences at p value ≤ 0.05 among treatments in each species

indices. Even 0.25-g and 0.36-g treatments involved significant germination decreases, by 26% and 46%, respectively. The germination percentage of *O. sativa* was affected by all three treatments with similar decrease values compared with the control (23% to 31%).

Root and shoot length of *E. oryzoides* were influenced by 0.36-g treatment with a reduction of 70% and 42%, respectively, while SVI was inhibited also by 0.25 g of leaves (53% and 69%).

The root growth and SVI in *O. sativa* showed an increasing reduction shifting from 0.25 g to 0.50 g (24% to 82% and 49% to 71%, respectively) while shoot elongation was not significantly affected (p value = 0.09).

All treatments were not able to affect the MGT in both species (p values > 0.05).

NMR and UPLC-HR-MS analysis

Aqueous extract of *L. multiflorum* leaves was characterized by mean of a combined analytical approach based on NMR spectroscopy and UPLC separation coupled with high resolution mass (HR-MS) analysis that has been already reported for characterization of plant extracts (Amigoni et al. 2017; Palmioli et al. 2019). In particular, UPLC separation was mainly targeted to the qualitative identification of polyphenols and secondary metabolites, whereas NMR spectroscopy data were complementary used for primary and secondary metabolites identification and quantification, including non-ionizable compounds. The chromatographic trace extracted at 320 nm, the characteristic absorbance of polyphenols, and ^{1}H-NMR profile with signal attribution of aqueous extract of *L. multiflorum* leaves were reported in Fig. 1 a and b, respectively. Detailed spectrometric HR-MS data used for compound identification are reported in Table 3. Overall, data analysis allowed the identification of 35 compounds, including several polyphenols, glycosyl flavonoids and glycosyl terpenoids. Among them, we clearly identified protocatechuic acid, 5-*p*-coumaroylquinic acid, apigenin and naringenin 6,8-di-*C*-glucoside and different glycosides of kaempferol and isorhamnetin. Moreover, we detected glycosyl terpenoids such as blumenol C-9-*O*-(2'-*O*-β-glucuronosyl)-β-glucoside, also known as blumenin, nor-isoprenoid trihydroxymegastigmane-4,7-dien-3-one-9-*O*-β-glucoside, also known as sauroposide, and the ubiquitous monoterpenoid lactone loliolide (Fig. 1a). In addition, ^{1}H NMR profile (Fig. 1b) showed also the presence of several amino acids, choline, γ-aminobutirric acid and different organic acids and polyphenols, including shikimic and gallic acids. After the

Table 2 Germination indices measured on filter paper for *E. oryzoides* and *O. sativa* under the effect of different quantity of *L. multiflorum* powdered leaves

Species	Powdered leaf quantity (g)	Germination (%)	MGT	Root length (mm)	Shoot length (mm)	SVI
O. sativa E. oryzoides	0.00	95.0 ± 5.8 a	5.4 ± 0.2 a	51.7 ± 13.0 a	28.5 ± 8.8 a	7552 ± 1595 a
	0.25	70.0 ± 9.1 b	6.0 ± 2.0 a	50.3 ± 25.5 a	22.3 ± 0.7 ab	3526 ± 937 b
	0.36	50.0 ± 8.2 c	5.8 ± 0.1 a	15.7 ± 8.3 b	16.5 ± 3.7 b	2317 ± 943 b
	0.50	0.0 ± 0.0 d	n.d.	n.d.	n.d.	n.d.
	F	141.8	0.4	5.6	4.7	20.9
	p value	0.000*	0.7	0.03*	0.04*	0.000*
	0.00	97.5 ± 5.0 a	5.0 ± 0.0 a	45.5 ± 10.0 a	18.2 ± 4.6 a	6205 ± 1586 a
	0.25	67.6 ± 20.0 b	5.5 ± 1.6 a	34.6 ± 2.2 b	22.5 ± 3.7 a	3151 ± 1215 b
	0.36	67.5 ± 19.0 b	5.3 ± 0.1 a	17.2 ± 2.7 c	12.7 ± 2.1 a	2037 ± 651 b
	0.50	75.0 ± 13.0 b	5.3 ± 0.4 a	8.2 ± 2.1 c	14.7 ± 8.3 a	1790 ± 893 b
F		5.6	0.2	38.1	2.7	12.6
p value		0.001*	0.8	0.000*	0.09	0.001*
Interaction species × treatment						
F		35.9	0.02	1.0	1.9	0.5
p value		0.000*	0.9	0.4	0.2	0.6

Values are mean ± standard deviation, asterisk and different letters indicate statistically significant differences at *p* value ≤ 0.05 among treatments in each species

manual identification of compounds, specific library was built using the simple mixture analysis tool implemented in the MestReNova 14.1 software. Simple Mixture Analysis allows for the simultaneous quantification of all metabolites contained in a complex mixture. The library developed with this approach is available as .exp. files (Palmioli and Airoldi 2019). Overall, the most abundant metabolites in crude extract were 2,3-butanediol (24.5 mM), succinate (19.8 mM), proline (12.95 mM), acetate (5.14 mM), alanine (4.91 mM), aminobutirric acid (4.07 mM), shikimic acid (2.95 mM), protocatechuic acid (2.92 mM) and lactate (2.34 mM).

Discussion

Till now, the effects of *L. multiflorum* phytotoxic activity were studied on the main grown crops, especially on rice (Li et al. 2008a, b; Lehoczky et al. 2011; Jang et al. 2018a, b), while the negative impact on weeds was only partially investigated (Vitalini et al. 2020; Jang et al. 2018b).

Our results confirmed the inhibitory action of *L. multiflorum* against rice weeds (Vitalini et al. 2020; Jang et al. 2018b). Furthermore, the obtained data supplemented the previous information on the effectiveness of *L. multiflorum* used as a cover crop. In particular, its leaves were able to significantly decrease the *E. oryzoides* germination, in both performed bioassays, unlike inflorescences, stems and roots (Vitalini et al. 2020). However, similarly to these organs, *L. multiflorum* leaves also significantly reduced the *E. oryzoides* seedling vigour. As it often happens, the roots were the vegetative structures most affected by the treatments (Vitalini et al. 2020; Favaretto et al. 2018).

In general, the phytotoxic effect was found to be dose- and species-dependent with a higher susceptibility of *E. oryzoides* compared with *O. sativa*. These data support the hypothesis based on the real-world farming environment experiences collected from some rice farmers, pioneers in the Italian organic rice sector (Orlando et al. 2020). The differences found between *E. oryzoides* and *O. sativa* in the in vitro assays are at the basis of their agronomic practices (Orlando et al. 2020). *L. multiflorum* has a greater impact on *E. oryzoides* than *O. sativa* in their early growth stages providing some competitive advantages to rice whose lower density, also evidenced by some in vitro treatments, is faced by farmers with a more abundant seed sowing. So, all in all, *L. multiflorum*, used as a cover crop before rice cultivation, can give a valuable contribution to the organic or low-input management of weeds. As a result, it may be expected that in the rice fields, the *L. multiflorum* residues can release compounds with a selective effect and, consequently, a potential role in weed control (Tabaglio et al. 2013). Considering the results reported by Li et al. (2008b), it is also possible to suppose that, among the decomposition products of *L. multiflorum* residues, there are some rice stimulators whose activity, mediated by soil

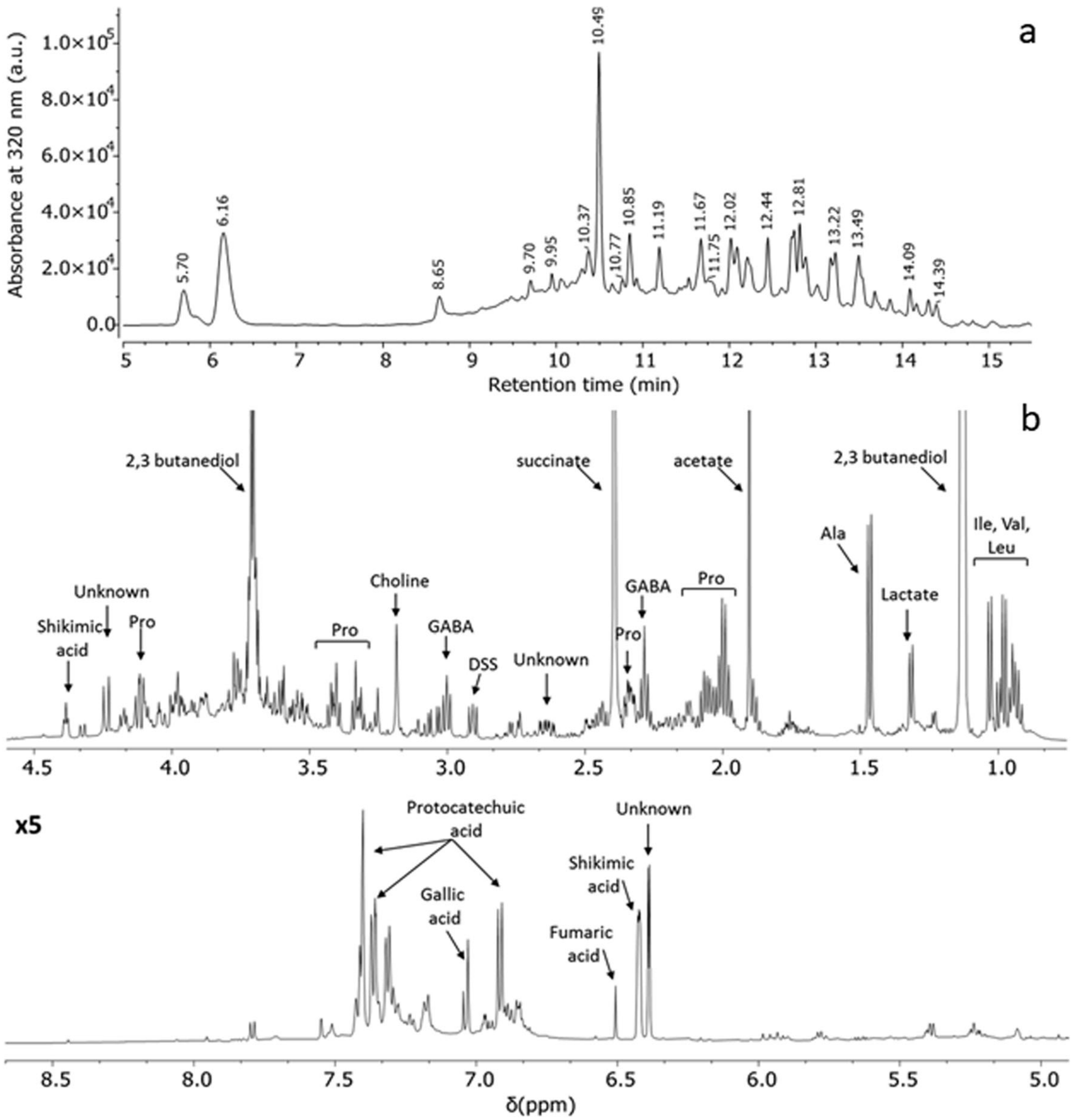

Fig. 1 UPLC separation trace extracted at 320 nm (**a**) and ^{1}H-NMR profile (**b**) of freeze-dried leaf extract from *Lolium multiflorum* dissolved in D_2O at a concentration of 10 mg/mL (DSS 0.5 mM, pH 7.4, 25 °C)

microorganisms, promote the growth of rice seedlings. In addition, it seems that *E. oryzoides* can be more susceptible to the *L. multiflorum* than *O. sativa,* at least in the administered quantities, due to the smaller size of its seeds (Synowiec et al. 2017).

In any case, the observed phytotoxic effect of *L. multiflorum* aqueous extract suggested that its leaves contained inhibitor compounds. Some of them have been reported as allelochemicals. For example, 2,3-butanediol identified in the *Caragana intermedia* Kuang and H.C.Fu root aqueous extract was considered as one of the allelochemicals for *Medicago sativa* Linn. through a concentration-dependent effect (Chen et al. 2017). Protocatechuic and gallic acids were among the main allelopathic compounds isolated from the aqueous extract of *Delonix regia* (Hook.) Raf. able to reduce the germination of the *Lactuca sativa* L. seeds more than 30% compared with the control (Li et al. 2010). Both compounds from the ethyl acetate fraction of the aqueous extract of *Merostachys riedeliana* Rupr. leaves showed also inhibitory effects on *Leucaena leucocephala* (Lam.) de Wit (Jose et al. 2016). Gallic acid identified in leachates of bark, fresh leaves and leaf litter of different *Eucalyptus* species and *Picea schrenkiana* Fisch. et Mey. significantly decreased the seedling growth of *Phaseolus mungo* L. and of the same *P. schrenkiana*, respectively (Li et al. 2010). In the rhizosphere soil of *Ageratum conyzoide* L., gallic acid helped to inhibit the *O. sativa* growth in terms of root length, shoot length and seedling weight (Li et al. 2010). The shikimic acid

Table 3 UPLC/HR-MS data for the major extract components identified in the aqueous extract obtained from *Lolium multiflorum* leaves

#	RT (min)	ID	Name	Molecular formula	Monoisotopic mass	HRMS (+) $[M+H]^+$	Abs. Error (ppm)	HRMS (−) $[M-H]^-$	Abs. Error (ppm)	λ_{Abs} (nm)	MS^2 (+) (rel. int.)	MS^2 (−) (rel. int.)
1	5.70	PCA	Protocatechuic acid	$C_7H_6O_4$	154.0261	155.0330	3.24	153.0190	2.02	260, 294	111 (100), 93 (85), 137 (27), 65 (20)	109 (100)
2	6.16	UNKN	Unknown			322.0538		320.0408		254, 320	322 (100), 169 (74), 276 (48), 258 (36), 286 (33)	153 (100), 166 (88), 320 (62), 122 (44), 109 (23)
3	6.71	Maltol	Maltol	$C_6H_6O_3$	126.0311	127.0384	4.53	–	–	273	–	–
4	8.27	Trp	Tryptophan	$C_{11}H_{12}N_2O_2$	204.0891	205.0964	3.70	203.0827	0.33	272, 279, 288	188 (100), 146 (39)	116 (100), 159 (35), 72 (35), 74 (32), 142 (28)
5	8.64	UNKN	Unknown			306.0583		304.0460		255, 296, 336		
6	10.40		Naringenin 6,8-di-*C*-glucoside	$C_{27}H_{32}O_{15}$	596.1736	597.1793	3.44	595.1676	1.22	251, 322	–	355 (100), 385 (92), 415 (29), 96 (14), 313 (12)
7	10.49	UNKN	Unknown			322.0538		320.0407		254, 305, 314	322 (100), 169 (95), 258 (47), 286 (44), 304 (41), 276 (38), 294 (33)	153 (100), 166 (86), 320 (57), 122 (43), 109 (23)
8	10.85	Vicenin 2	Apigenin 6,8-di-*C*-glucoside	$C_{27}H_{30}O_{15}$	594.1590	595.1625	4.77	593.1508	0.95	270, 325	325 (100), 379 (99), 409 (70)	353 (100), 473 (74), 383 (60), 593 (42)
9	11.18	UNKN	Unknown			306.0586		304.0460		259, 322		
10	11.67		Kaempferol glucoside-glucoronide	$C_{27}H_{28}O_{17}$ $C_{15}H_{10}O_6$ aglycone	624.1332 286.0472	625.1374 287.0541	4.07 3.17	623.1245 285.0408	1.33 1.19	267, 338	287 (100)	285 (100)
11	12.00	5-pCoQA	5-pCoumaroyl quinic acid	$C_{16}H_{18}O_8$	338.1007	339.0996	3.88	337.0925	1.21	271, 305	–	191 (100)
12	12.20	UNKN	Unknown	$C_{15}H_{17}O_{13}$	405.0675	406.0732	2.24	404.0613	3.88	255, 335	304 (100), 286 (37), 388 (33)	–
13	12.27	Rutin	Quercetin-3-*O* rutinoside	$C_{27}H_{30}O_{16}$ $C_{15}H_{10}O_7$ aglycone	610.1539 302.0421	611.1585 303.0493	3.55 1.93	609.1466 301.0279	0.38 0.73	265, 315–350	303 (100)	300 (100)
14	12.44		Apigenin glucoside-glucoronide	$C_{27}H_{28}O_{16}$ $C_{15}H_{10}O_5$ aglycone	608.1383 270.0534	609.1425 271.0592	4.05 3.32	607.1287 269.0457	2.79 0.51	265, 309–350	271 (100)	269 (100)
15	12.70		Diosmin glucoside-glucoronide	$C_{28}H_{30}O_{17}$ $C_{16}H_{12}O_6$ aglycone	638.1483 300.0634	639.1531 301.0695	3.82 3.92	637.1410 299.0562	0.36 0.14	265, 290, 320–365	331 (100)	329 (100)
16	12.76		Dillenetin 5-glucoside-7-glucuronide	$C_{29}H_{32}O_{18}$ $C_{17}H_{14}O_7$ aglycone	668.1594	669.1632 331.0802	4.43 3.21	667.1505 329.0670	1.70 1.12	265, 290, 320–365	331 (100)	329 (100)
17	12.81		Kaempferol 3-neohesperidoside	$C_{27}H_{30}O_{15}$ $C_{15}H_{10}O_6$ aglycone	594.1579 286.0472	595.1635 287.0543	3.79 2.64	593.1483 285.0404	4.32 0.20	265, 290, 320–340	287 (100)	284 (100), 285 (60)

Table 3 (continued)

#	RT (min)	ID	Name	Molecular formula	Monoisotopic mass	HRMS (+) $[M+H]^+$	Abs. Error (ppm)	HRMS (−) $[M\text{-}H]^-$	Abs. Error (ppm)	λ_{Abs} (nm)	MS^2 (+) (rel. int.)	MS^2 (−) (rel. int.)
18	12.99	Sauroposide		$C_{19}H_{30}O_9$	402.1895	–	–	401.1813	0.94	–	–	401 (100), 101 (21), 71 (15), 113 (11), 221 (10)
19	13.10	Blumenin	Blumenol C 9-*O*-(2′-glucuronosyl)glucoside	$C_{25}H_{40}O_{13}$ $C_{13}H_{22}O_2$ aglycone	548.2463 210.1614	549.2522 211.1688	3.63 2.21	547.2369 209.1547	2.86 0.10	–	135 (100), 193 (85), 211 (64), 109 (59), 175 (57), 119 (36), 357 (6)	547 (100), 113 (84), 175 (11), 371 (8)
20	13.17	Calendoflavoside	Isorhamnetin 3-neohesperidoside	$C_{28}H_{32}O_{16}$ $C_{16}H_{12}O_7$ aglycone	624.1696 316.0578	625.1736 317.0647	4.35 2.69	623.1611 315.0514	1.08 1.25	265, 290, 328	317 (100)	314 (100), 315 (100
21	13.23		Kaempferol-glucoside	$C_{21}H_{20}O_{11}$ $C_{15}H_{10}O_6$ aglycone	448.1000 286.0458	449.1059 287.0543	4.34 2.42	447.0916 285.0397	3.79 2.54	265, 290, 326	287 (100)	284 (100), 285 (28)
22	13.48		Kaempferol-glucoside	$C_{21}H_{20}O_{11}$ $C_{15}H_{10}O_6$ aglycone	448.1000 286.0458	449.1060 287.0544	4.14 2.32	447. 0915 285.0402	4.05 0.94	265, 290, 326	287 (100)	284 (100), 285 (28)
23	13.55		Isorhamnetin-glucoside	$C_{22}H_{22}O_{12}$ $C_{16}H_{12}O_7$ aglycone	478.1106 316.0578	479.1165 317.0649	3.95 2.02	477.1029 315.0503	1.96 2.37	265, 290, 328	317 (100)	314 (100)
24	13.68		Isorhamnetin-glucoside	$C_{22}H_{22}O_{12}$	478.1106	479.1165	3.95	477.1018	4.22	265, 290, 328	317 (100)	314 (100)
25	13.82		Loliolide	$C_{11}H_{16}O_3$	196.1094	197.1163	4.85	–	–	–		
26	14.08		Kaempferol-acetylglucoside	$C_{23}H_{22}O_{12}$ $C_{15}H_{10}O_6$ aglycone	490.1170 286.0458	– –	– –	489.1034 285.0406	0.97 0.34	265, 290, 326	–	285 (100), 284 (84)
27	14.16		Isorhamnetin-malonyl glucoside	$C_{25}H_{24}O_{15}$ $C_{16}H_{12}O_7$ aglycone	564.1110 316.0578	565.1162 317.0648	4.59 2.40	– –	– –	265, 290, 328	317 (100)	
28	14.39	UNKN	Unknown			254.1006		252.0877		270, 317	208 (100)	208 (100), 164(72), 152 (39), 107 (22)

is the common precursor for the synthesis of different phenolic compounds (e.g. coumarins, terpenoids, phenolic acids) implicated in plant allelopathy observed in both natural and managed ecosystems (Ravazi 2011; Favaretto et al. 2018).

In conclusion, confirmation of the different phytotoxic effects of *L. multiflorum* on *E. oryzoides* and *O. sativa* strengthens the practical knowledge of farmers coming from long-standing direct experiences. Even though the laboratory experiments provide only preliminary results on the potential species-specific relationship and the field conditions can interfere with the dynamics observed in controlled environment, the study data provided evidences to support the use of *L. multiflorum* as a cover crop to reduce the weed incidence and obtain better yields. Its green mulching seems to be a viable and alternative strategy among agroecological practices aimed to improve the sustainability of the crop production.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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Chapter III

In order to deepen the studies presented in the previous chapters, it was essential to evaluate the environmental impacts of the main organic rice cultivation techniques. A study applied the LCA methodology to the cultivation and productive scenarios from chapter 1. This study is currently under review in an international sector journal. The main highlights concern:

- The study compares four alternative agricultural practices and two production potential levels observed during three-year monitoring on 10 farms in the study area. The assessed environmental performance of organic rice production took into account two productive levels recorded for the study area 3.91 t/ha (Q2) and 5.65 t/ha (Q3), and thus a range of yield variability consistent with the data shown in literature by similar studies.
- The results suggest a considerable potential of organic rice production to mitigate its impact on natural resources, depending on the chosen agricultural practices. In particular, six LCA indicators showed a potential reduction of over 40%, shifting from the worst-performing management to the better one.
- The discrepancy in results while assessing environmental impact is mainly due to the different researchers' management patterns.
- The "cover crop-based" systems showed the more considerable impact decrease potential, in correspondence of yield improvements, and involves the "good practices" (i.e. use of cover crop and leguminous species, use of input internal to the farm) that are supported by the public authorities and by the policies for the sustainability.
- LCA outcomes point out the need to integrate this tool, suitable for the assessment of the impact, with others, suitable for the evaluation of the environmental benefits and ecosystem services, in order to obtain a reliable, systemic and integrated assessment of the sustainability of farming systems and its management systems.

LCA methodology to evaluate environmental impacts of different organic rice management for rice production in Italy.

--Manuscript Draft under revision from the authors--

Types of papers: Original paper

Title: Environmental impacts of different management and productive scenarios for organic rice in Italy.

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Abstract

Rice cultivation has a key role in food security worldwide; on the other hand, it has a high potential impact on the environment and human health, mainly due to the extensive pesticides use and greenhouse gas emissions caused by flooded cultivation. Here we investigate how the transition toward organic agriculture can improve the environmental performance of rice farming according to the actual European sustainable food production strategy. Through LCA methodology, here we show for the first time a study that aims to evaluate the variability of the environmental impacts and the mitigation potential of four management strategies suitable for organic rice production in North Italy and two production potential levels observed during three year monitoring on 10 farms in the study area. The LCA analysis includes the wide range of agronomic realities that characterize this farming system, assessing the variation in environmental performance by exploring eight plausible and possible scenarios for organic rice. Results suggest a considerable potential of organic rice production to mitigate its impact on natural resources, depending on the chosen agricultural practices. In particular, six LCA indicators showed a potential reduction of over 40%, shifting from the worst-performing management to the better one.

Finally, the large variability of climate change impacts assessed, both in this study and in literature, is due to the large variability in yield and available patterns of agricultural practices. Today, farmers could reach acceptable yield values thanks to more efficient management than in the past. The acknowledgement for that performances relates to the development of the farmers' know-how and to the productive improvement connected to the long-term processes which characterize the organic systems (e.g. generation of soil fertility based on biological fertility and stable humus components; lowering of weeds pressure through the gradual introduction of other crops in rotation).
Keywords: Oryza sativa; organic rice; organic farming; LCA; environmental assessment; GHG emissions

1. Introduction

Food production addresses one of the most essential and basic human needs. That is even more true considering rice, a crop with a key role for food security in many countries worldwide (Seck et al., 2012). On the other hand, rice farming has a high potential impact on the environment and human health, due to the extensive pesticides use, in particular herbicides, that occurs when the economic growth and rising labour cost lead to a progressive giving up of hand weeding practice (Naylor, 1994). The rice crop is significantly affected by the crop-weed competition that, in the absence of agrochemicals, is considered the leading cause of yield variability and the main constraint of realizing potential yield (Delmotte et al., 2011; Hazra et al., 2018). It follows that rice was identified as the single most important crop in terms of sold and consumed agrochemicals (Woodburn, 1990).

In Italy, the rice sector is mainly based on high-input monoculture, and the highest degradation of water quality due to pesticides and herbicides pollution is in the Northern rural areas (i.e. between Piedmont and Lombardy regions) (ISPRA, 2018), where 94% of the national rice area is placed (ISTAT, 2018). Thus about 49% of the European rice cultivations is concentrated (FAO, 2016). In this context, the transition toward organic agriculture can improve the environmental performance of rice farming. Nowadays, that transition is also encouraged by the European sustainable food production strategy "Farm to Fork," which is the core of the European Green

Deal aiming to make food systems fair, healthy and environmentally friendly, encouraging the expansion of the organic agriculture sector (European Commission 2020).

In general, organic farming is capable of reducing the environmental impact of agriculture by avoiding the use of synthetic compounds (e.g. fertilizers, pesticides) and by promoting practices (e.g. crop rotation, leguminous cultivation, organic fertilizers, green manure crops, green mulching.) able to increase the soil carbon stock, and prevent the indirect environmental impacts due to the industrial production of inputs (Acuna et al. 2018). Organic agriculture produces biodiversity, with increases of abundance and species richness observed for birds, mammals, invertebrates and flora (Hole et al., 2005), shows higher economic values concerning some ecosystem services (Sandhu et al. 2008), and leads to a decrease of nitrate concentration into the water (Honisch et al., 2002). For rice, in particular, the organic system was observed able to increase the soil carbon storage capacity (Komatsuzaki and Syuaib, 2010) and organic matter content, facilitating the soil preparation (Mendoza, 2004), and promoting the ecological succession and temporal heterogeneity of the macrophyte communities into the soil (MartínezEixarch et al.; 2017).

The Life Cycle Assessment (LCA) is an approach to quantify the environmental impacts of products and/or services, and it is increasingly a key tool to support the transition towards more sustainable production patterns. Even if initially developed for industrial processes, LCA was more and more applied to agricultural systems over the years. However, results from LCA reveals peculiar features of organic rice cultivation compared to those of other herbaceous organic crops. Aguilera et al. 2015 studied the global warming potential of 38 pairs of conventional and organic herbaceous cropping systems in Spain (functional unit: 1 kg product), showing the reduction of emission by 36-65% organic management was assessed, except for rice. Unlike the other crops, organic rice showed an increase of emission by 8%, compared to conventional rice. The authors highlighted that the rice cultivation in flooded fields differs from other crops because the greenhouse gases (GHG) balance mainly focuses on the methane emissions generated in water-saturated conditions for the anaerobic decomposition of organic matter. The organic rice involves incorporating the soil of organic manure and crop biomass, which, together with the lower yield, increases the methane emissions for a share that the carbon sequestration could not overcome and thus was not able to offset. Hokazono and Hayashi (2012), Hokazono et al. (2009), and Bacenetti et al. (2016), comparing organic and conventional rice, came to similar results, respectively, in

Japan and North Italy, assessing an overall higher impact on organic management. Similarly, the study of Blengini and Busto (2009), carried out in North Italy, pointed out that despite the organic rice can decrease the impact per unit of cultivated area, this benefit declines when the product is a functional unit due to the yield decrease. Therefore, despite the above mentioned ecological benefit and positive externalities related to organic rice and organic agriculture, the LCA environmental evaluations, taking into account the environmental impacts, suggest that organic rice farming needs to apply mitigation practices, on par with the conventional system, choosing among different management options those with the lower impacts.

However, few studies in literature address this issue, evaluating the variability of LCA outputs and assessing the mitigation potential among different management strategies available for organic rice growing. The comparative studies between organic and conventional rice generally show some limitations. Insufficient attention was on organic rice's existing range of practices and productive performances. Often, organic farming seems to be considered a unique system, based on applying a universal management recipe extendible, with a slight approximation, from one monitored farm to all the organic farms of the study area. Most of LCA carried out on organic rice assumes a limited variation and slight differences in agricultural practices, between farms or between the growing seasons, so much that it is enough to take into account few farms or one season to obtain data on management and yield (and thus environmental assessments), representative of the majority. Concerning the previously mentioned studies, Aguilera et al. (2015) considered three rice farms, characterized by shallow, productive performances (i.e. 2.48 t/ha d.m.), without providing details on crop management. Blengini and Busto (2009) considered the organic management adopted by only one rice farm and the resulting yield (i.e. 4.4 t/ha). Hokazono and Hayashi (2012) assessed the environmental impact based on the yield (3.37 t/ha) averaged on two different managements identified for organic rice. Bacenetti et al. (2016) took into account the average yield reached during one growing season in 19 fields (i.e. 4.5 t/ha at commercial moisture 14%) by only one rice farm, and the related management, based on mechanical weed control (i.e. four passages of harrow) and large organic matter input (i.e. incorporation into the soil of both compost and cover crop biomass).

However, Bell et al. (2008) pointed out that organic agriculture is more than a process of recipe adoption: the universal practices and standard protocols are unsuitable for managing the complexity of organic cropping systems, affected by cumulative effects long-term dynamics.

Concerning rice, Orlando et al. (2020), monitoring 50 organic rice fields belonging to 10 farms during the three-year study in North Italy, reported the adoption by the farmers of knowledge-intensive adaptive management strategies. The authors identified three main functional principles underlying these strategies: weed control involves the mechanical action to exploit phytotoxic effects (Vitalini et al., 2020a; Vitalini et al., 2020b). Each identified strategy was not a universal recipe but involved a wide range of operative variants, maintaining the same functional principle but shaping the agronomic practices to the case-specific needs (i.e. season-specific and site-specific). Furthermore, a wide range of productive performance occurred (i.e. grain yield at commercial moisture 14%: in middle-quartile 4 t/ha, in upper-quartile 6 t/ha). The organic rice yield was affected by many sources of variability, such as the history of the field (i.e. previous crop rotation) and the farmer's know-how. The farmer experience and ability to identify the strategy fitting the site-specific cultivation conditions and apply timely the related agronomic practices during the growing season were crucial aspects in determining the productive performance. All this results in a wide range of management and production scenarios.

In this context, Bacenetti et al. (2016) study are valuable since, even if it starts from the cropping system adopted by only one farm, it compares the current management practices (i.e. baseline scenario) with five alternative management practices. The authors assumed for the LCA the introduction of aeration during the cultivation period and replacing compost with other organic manurings, analyzing the related mitigation potential in environmental impact. The changes in fertilizer management reduced the environmental impacts from 13% up to 51% depending on the impact categories.

Regarding management strategy, practices, and productive performance, differences among different scenarios could explain discrepancies between the comparative studies, organic vs conventional, carried out on rice with LCA methodology.

He et al. (2018) found for organic rice cropping systems in China lower impacts (functional unit: 1 kg product) concerning non-renewable energy depletion, water depletion, acidification potential, eutrophication potential, aquatic toxicity potential and human toxicity potential, and recommended the organic rice farming as sustainable agricultural practices, in comparison with conventional. These results are based on data collected from 98 farms, thus considering a wide range of farm realities and management systems practised for 5, 10 and 15 years, with the related average yield (i.e. 5.3, 6.0 and 6.1 t/ha, respectively). Conversely, Hokazono and Hayashi (2012) found for organic rice worst environmental performance than conventional rice, for the following impact categories: non-renewable energy depletion, acidification potential, eutrophication potential; and similarly Bacenetti et al. (2016) for the following impact categories: mineral and fossil resource depletion (i.e. similar to non-renewable energy depletion category), terrestrial acidification (i.e. similar to acidification potential category), freshwater and marine eutrophication (i.e. components of the eutrophication potential), and human toxicity potential.

In contrast with the current literature, Yodkhum et al. (2017) showed that the GHG emissions of organic paddy rice were considerably lower than conventional, taking into account data collected from sixteen farms in Thailand and information retrieved from different local sources

(Don-Chiang Organic Agricultural Cooperative, Mae-teang district, Chiang Mai province, Office of Agricultural Economics, Ministry of Agriculture and Cooperatives). The authors explained the highest environmental performance found for organic rice with the lower use of organic fertilizers, the lower diesel fuel consumption and the different water regime during the cultivation period (i.e. rain-fed and deep water, instead of continuous flooding), compared to the conventional system and other organic systems considered by previous studies.

In this context, the present study aims to evaluate through LCA the variability of the environmental impacts and the mitigation potential of four management strategies suitable for organic rice production in North Italy. The study compares four alternative patterns of agricultural practices and two production potential levels observed during three-year monitoring on 10 farms in the study area (Orlando et al., 2020). The study wants to make the LCA analysis inclusive of the wide range of agronomic realities that characterize this farming system, assessing the variation in environmental performance by exploring eight plausible and possible scenarios for organic rice. Figure 1 highlight briefly the topic and result of the article. Figure with colour

Figure 1 highlights the topic and result of the article briefly.

2. Materials and methods

2.1 Study area and data collection

The study area was the North Italian rice cultivation district, where during three growing seasons (i.e. 2016, 2017, 2018), the research considered a total number of 50 rice paddy fields. The fields belonged to ten organic farms located in the two crucial Italian rice provinces: Vercelli and Pavia

province (in Piedmont and Lombardy region, respectively), covering 67% of the national production (Istat, 2016).
The monitoring involved ten farmers identified as local pioneers of organic rice farming during the participatory research by Orlando et al. (2020). The latter study aimed to fill the knowledge gap about the management strategies and productive performance reachable by growing rice with an organic system, and the present work started from the author's conclusions to define the scenarios described in the following 2.3 and 2.4 sections.
A wide range of cultivation conditions characterized the 50 fields (see Annex A in Supplementary Material):

- 62% and 38% were in Pavia and Vercelli provinces, respectively (fewer fields in Piedmont where the organic rice sector is a younger reality);
- 44% characterized by silty-loam soil and 12% by silt soil (mainly associated with fields in Vercelli province, whose soils are characterized by a higher percentage of silt), while 40% was characterized by sandy-loam and 4% by loamy-sand soil (mainly associated with fields in Pavia province, whose soils are characterized by a higher percentage of sand);
- 30% were monitored in the 2016 growing season, 44% in 2017 and 26% in 2018;
- 46% was managed by farmers with eight or more years of experience in organic farming (most farmers of Pavia province), while 54% of farmers started to grow organic rice between 2014 and 2015 (most farmers of Vercelli province).

For all the fields, information concerning the agricultural practices, agronomic inputs, and the resulting grain yield (ton per hectare at 14% of commercial moisture) was collected through face-to-face farmer interviews and field surveys; in the beginning, during and at the end of the crop cycle.
Background data for the production of seeds (rice, ryegrass and vetch), fuel, organic manure, tractors and agricultural machines were from the Ecoinvent database Database v.3.6 (Althaus et al., 2007; Frischknecht et al. 2007; Jungbluth et al., 2007; Nemecek and Käggi, 2007; Spielmann et al., 2007). For a sample of ten fields, the fuel consumptions and time of intervention were directly measured during field surveys through a stopwatch and a graded portable tank of fuel, respectively. This sub-sample of fields were representative of some agronomic interventions underlying the

management strategies that were taken into account (see section 2.4). The data were used to validate the fuel consumption values assessed with the support of the Ecoinvent database Database v.3.6. for the less common machinery used in the organic rice field (e.g. minimum tillage machinery). The production of straw was considering a harvest index of 0.45 (Boschetti et al., 2006)

2.2 Life Cycle Assessment

The Life Cycle Assessment (LCA) aim to evaluate the environmental impacts of the organic rice cropping system, considering eight scenarios, given by four management strategies combined with two production levels, as shown in table 1. The scenarios are better detailed in sections 2.3 and 2.4.

Table 1 Environmental impact (EI) categories computed in eight scenarios for organic rice cultivation, given by four management strategies (CC_v1 = green mulching with broadcast rice sowing; CC_v2 = green mulching with dry period; SD = stale seedbed in dry paddy; SF = stale seedbed in flooded paddy), each one combined with two production levels (Q2 = middle yield, 3.91 t/ha; Q3 = upper yield, 5.65 t/ha).

The LCA was performed with the support of SimaPro Software 9 and following the ISO 14040/44 methodology (ISO, 2006) and the Environmental Product Declarations (EPD) guidelines defined for "Arable Crops" (Environdec, 2014). The midpoint ILCD method (Wolf et al., 2012) considered the impacts categories described in table 1, recognized as the most representative agricultural systems (Guinée and Lindeijer, 2002). The functional unit (FU), chosen as a reference unit in LCA, was the most common in literature: the rice grain production, expressed as 1 ton, at commercial moisture of 14%, to quantify the environmental performance with results comparable with similar studies on rice. Concerning the system boundaries, the study performed a "from cradle to farm gate" assessment, considering the following processes: the extraction of the raw materials (e.g. minerals, fossil fuels and metals), the production of agricultural inputs (e.g. seeds, fertilizers, tractors, machines, lubricant), the supply of inputs to the farm (e.g. transport), the use of agricultural inputs (e.g. fertilizer application, diesel fuel and lubricant consumptions, tire abrasion, and the related pollutant emissions), the maintenance and final disposal of machines, the grain and straw production, the emissions of N and P compounds due to fertilizers applications, field flooding (methane), and fuels combustion. The LCA was carried out following the setting already used for organic rice grown in the same area of Bacenetti et al. (2016) concerning this and the emissions from fertiliser application and organic matter decomposition.

2.3 Scenarios: production levels

The organic rice yield data published by Orlando et al. (2020) was considered since the vast number of monitored fields (i.e. 50), with heterogeneous features, supply a representative framework concerning the possible production trend drawn by organic rice farming in the study area. The yield dataset showed normal distribution and high variability, with mean values of 3.91 t/ha and 5.65 t/ha respectively in the middle (Q2) and upper (Q3) quartile. Therefore, LCA analysis took into account two plausible and probable production levels. i) the currently more common situation in the study area (i.e. middle yield level Q2) and ii) an improvement scenario, in which the yield increase in the short-term, mainly thanks to the crop rotation adoption and the development of the farmer's know-how and her/his adaptive management (with resulting decrease of yield losses due to weed incidence).

2.4 Scenarios: management strategies

The most promising management strategies, resulting from the fields monitoring, were based: i) on mechanical weeding, with stale seedbed and shallow tillage, performed in dry paddy field (i.e. strategy: stale seedbed in dry paddy; SD) or flooded paddy field (i.e. strategy: stale seedbed in flooded paddy; SF); ii) on weeds control performed through green mulching from a cover crop, and the flooding of the resulting biomass close to the sowing (strategy: green mulching with broadcast rice sowing; CC_v1) or after a post-sowing dry period (i.e. strategy: green mulching with dry period; CC_v2). The LCA took into account the four crop management options. The agricultural practices, agronomic input and flooding days' data are in table 2 (SD and SF) and table 3 (CC_v1, CC_v2) and described below.

Table 2. Patterns of agricultural practices concerning the "stale seedbed in dry paddy" (SD) and the "stale seedbed in flooded paddy" (SF) strategies, both based on mechanical weed control.

"Stale seedbed in dry paddy" (SD): the strategy based on the weeds' mechanical control and the well-known technique of the stale seedbed in the dry field (Ferrero, 2003). After the harrowing follows a superficial passage with comb harrow, carried out both in pre-and post-sowing. The number of passages with comb harrow varies on the weed incidence until seven steps. The rice sowing is in-row on dry land. The sowing depth (5 cm) and the post-sowing dry period (on average 23 days) are adopted to comb harrow operability and avoid rice seedlings damage. The strategy is feasible in intermittent water access (i.e. constrained by the water supply calendar established by the local authority), but it is not so suitable for soil with a firm texture.

"Stale seedbed in flooded paddy" (SF): contrary to the SD strategy, after the harrowing, the rice paddy field is subsequently flooded for an average of 14 days before sowing. So, weed eradication occurs in water through minimum tillage (i.e. two interspersed passages). The farmers used innovative machinery for this operation and realized modifying existing ones (i.e. adding tines to a bar generally used for field levelling). After that, the broadcast sowing of rice occurs in water. The weed control is performed mechanically and by the "puddling" effect (Bhagat et al., 1996, physical obstacle and anaerobic conditions provided by mud). This strategy requires flexible access to the irrigation water supply, and it is not so suitable for loose soil with fast drainage.

The agricultural practices, agronomic input and flooding days' data are CC_v1 CC_v2 are in table 3 (CC_v1, CC_v2) and described below.

Table 3 Patterns of agricultural practices concerning the "green mulching with broadcast rice sowing" (CC_v1) and the "green mulching with dry period" (CC_v2) strategies, both based on weed control through the use of cover crops and their biomass.

"Green mulching with broadcast rice sowing" (CC_v1): a cover crop mixture (i.e. graminaceous, such as Lolium multiflorum, and leguminous, such as Vicia sativa) sown before rice, and the weed control based on complex dynamics that involve the cover crop competition, the green mulching effect, the allelopathic relationships between weeds and the sowed species (Vitalini et al., 2020a; Vitalini et al., 2020b), and the toxic effects of the organic acids developed as a consequence of the cover crop biomass fermentation. The broadcast sowing of rice is on dry land and the standing cover crop. Immediately after, chopping the cover crop and the field flooding, activating the fermentation processes. This flooding lasts five days and follows a dry period (on average 12 days). Timely water management is a crucial element for the success of this strategy. Then, flexible access to the water supply is required.

"Green mulching with dry period" (CC_v2): this strategy is a variant of the CC_v1 system, developed by the farmers to minimize the negative impact of the organic acids on rice germination. The main differences are the following: i) the rice is sown in-row, on dry land and the standing cover crop; ii) instead of flooding, a post-sowing dry period follows (on average 30 days), at the end of which the cover crop chopped, and the field flooded. This flooding lasted eight days, followed by a dry period (on average 12 days). The postponing of these operations later in the season, compared to CC_v1 strategy, shifts the fermentation period when rice is at the leaf development stage (3°- 4° leaves unfolded), instead of at the germination stage, and in the farmer's opinion, this could be able to reduce the adverse effects of organic acids on rice.

Among the four management strategies, some operations are in common: the field levelling and banks maintenance, and, even if carried out in different periods or at different depths, the ploughing and harrowing. On the other hand, the organic manuring differs i) the SD system is high-intensive in mechanical operations and often applied in soil characterized by higher sand percentage and faster mineralization rate. Therefore, it is good practice to integrate the soil fertility with an annual input of organic manure; ii) in the CC_v1 and CC_v2 systems, combining leguminous species as a cover crop and minimum tillage conservative practices, the organic manuring is planned every three years and the same occurs in SF systems: since usually the farmer, that chooses the SF strategy, decides to apply it year in, and year out, alternating with the CC_v1 or CC_v2 systems.

Finally, the irrigation schedules (i.e. cycles of flooding and dry periods) are in figure 2: all the strategies have in common a dry period in July, even if with different duration, and involved a non-irrigated pre-harvest period starting from about 20 August. On the other hand, the SD and CC_v2 systems are characterized by a long dry period compared to the other strategies.

Figure 2 Irrigation schedule describes the flooding cycles for the management scenarios, characterized by a rice row-sowing and post-sowing dry period (i.e. SD and CC_v2) or rice broadcast-sowing and subsequent flooding (i.e. SF and CC_v1).

3. Results and discussion

3.1 Comparison between the management strategies

Table 4 reports for organic rice production the environmental impacts, based on 11 impact categories, taking into account eight different scenarios, combining four management strategies and two production levels.

Table 4 Environmental impact (EI) categories referred to 1 t of paddy rice at commercial moisture, evaluated in eight scenarios for organic rice cultivation. Legend: Δ = absolute difference Q3-Q2; CC_v1 = green mulching with broadcast rice sowing; CC_v2 = green mulching with dry period; SD = stale seedbed in dry paddy; SF = stale seedbed in flooded paddy; Q2 = middle yield; Q3 = upper yield.

Focusing on the environmental impact category "climate change" (CC), figure 3 shows the contribution analysis in the four management systems. These data reveal some of the main differences among the agricultural strategies and are helpful in part to explain the values shown in table 4.

Figure 3 Percentage contribution of different sources of GHG emissions to climate change total value assessed for each management strategy (CC_v1 = green mulching with broadcast rice sowing; CC_v2 = green mulching with dry period; SD = stale seedbed in dry paddy; SF = stale seedbed in flooded paddy). Figure with colour.

Table 4 shows for the SD system the better environmental performance concerning the impact categories: CC, PM, ME, TA (fig. 3a, 3b, 3c, 3d) and TE (Annex B), while the worst one in terms of MFRD (fig. 3f).

Figure 2 For the environmental impact categories CC, PM, ME, TA, MFRD, HTc, the minimum, maximum, and mean value (the bottom, top and middle line of each box, respectively), evaluated for the four management strategies (i.e. CC_v1, CC_v2, SD, SF).

Figure 3 shows for SD management the lower percentage contribution to CC due to methane emissions (i.e. 50.2% vs a range of 52.6-60.7% assessed for the other systems) and emissions from fertilizers (i.e. 11.7% vs a range of 16.0-27.6% of the others), but the more significant contribution due to mechanical operations (i.e. 14% vs a range of 4.2-6.8% of the others) and depth sowing (i.e. 6.2% vs a range of 3.3-3.6% of the others). The lower environmental impacts are due to the incorporation of stabilized organic matter into the soil through organic fertilizers, instead of the green biomass used by the "cover crop-based" systems (CC_v1 and CC_v2), and to the shorter period of flooding (total 67 days), compared to SF (101 days) or CC_v1 (79 days) systems, thanks to the post-sowing dry period. That reduces the impact on natural resources, minimizing the negative phenomena associated with the anaerobic decomposition of organic matter in the rice paddy field. On the other hand, the SD system involves mechanical weed control, based on many passages of shallow tillage and depth sowing, leading to a higher quantity of consumed fuel.

The SF system follows second in place the SD strategy in the best environmental performances (Table 4), for what concern the categories: CC, PM, ME, TA (fig. 3a, 3b, 3c, 3d) and TE (Annex B), with lower impacts than CC_v1 and CC_v2 systems. The MFRD values, even higher than the "cover crop-based" strategies, are much lower than SD (fig. 3f), thanks to the few mechanical passages and the broadcast sowing. Figure 3 shows SF management the highest percentage contribution to CC due to methane emissions (i.e. 60.7% vs a range of 50.2-53.7% of the others), resulting from the extended flooding period that starts two weeks before sowing. Moreover, contrary to the SD system, moderate fuel consumption makes the mechanical operations responsible for 6.8% of the GHG emissions in the SF strategy. In SF management, the moderate use of fuel and organic fertilizer balances environmental impact due to the prolonged period of flooding. This strategy can obtain an adequate weed control, thanks to the combination of the mechanical action with targeted water management and the "puddling" effect, and at the same time to involve a low fuel consumption, close to "cover crop-based" systems but avoiding their environmental complications due to the fermentation of green biomass.

Table 4 shows for the "cover crop-based" systems the worst environmental performance for what concern the categories: CC, PM, ME, TA (fig. 3a, 3b, 3c, 3d) and TE (Annex B). However, for these categories, a noticeable potential for improvement is revealed, shifting from Q2 to Q3 level of production. SD and SF systems showed slight differences in the environmental performance between the two production scenarios (i.e. Q1 and Q2); CC_v1 and CC_v2 showed a more considerable difference and, thus, a wide variation range decreasing their environmental impacts the yield increase. Moreover, CC_v1 and CC_v2 showed a lower impact in terms of MFRD (fig. 3e). The low fuel consumption is due to the "sod seeding" (CC_v2) or broadcast sowing (CC_v1) and the replacement of mechanical weeding with the green mulching practice. On the other hand, this innovative and successful practice for weed control (Orlando et al., 2020) determined the lower environmental performance concerning the process of anaerobic fermentation of the flooded green mulching or the release of nitrogen compounds from the long-term decomposition of the leguminous biomass. No noticeable differences between CC_v1 and CC_v2 are in figure 3 regarding the percentage contribution of the GHG emissions sources to CC value. On the other hand, both systems showed higher percentages for the emissions from fertilizer (i.e. macro-nutrients from the cover crop biomass) than the SD and SF management (26.2-27.6% vs a range of 11.7-16.0% of the others). In absolute terms (table 4), the two "cover crop-based" systems have in most cases similar environmental performances, except for the categories: HTc (fig. 3f), OD and FEx (Annex B, figure bc and be), for which CC_v2 showed lower impact than CC_v1, probably concerning its more extended post-sowing dry period.

Concerning these environmental categories (i.e. HTc, OD, FEx), CC_v2 and SF systems moved within similar values, performing better than CC_v1 and SD strategies.

Finally, the results (Table 4) show that environmental impact values within a wide range of variation for organic rice, depending on the farmer's choices about water management, weed control strategy, and nutrients and organic matter replenishment plans. Moreover, the results suggest different environmental impacts, shifting from Q2 to Q3 productive level, with different sensitivities depending on the management strategy: the four systems showed different improvement potential for some important impact categories.

The management based on the mechanical weed control and external fertilizer input (i.e. SD) results globally in the best environmental performance. At the same time, the CC_v1 and CC_v2 systems showed the worst performance for most categories, even if they were based on good agricultural practices, such as the use of resources internal to the farm for the maintenance of soil fertility and the low soil disturbance through minimum tillage and the cover during the winter period. However, extensive literature established the multiple environmental benefits due to the use of winter cover crops (Dabney et al., 2001), leguminous plants (Stagnari et al., 2017) and green mulch (Thakur and Kumar, 2020). These practices are among the topics addressed by the European Innovation Partnership (EIP-AGRI; https://ec.europa.eu/) in order to foster competitive and sustainable farming, and they are supported by the local policy for agriculture in many countries and by FAO in the strategies of conservation agriculture (FAO 2011) or integrated soil fertility management (FAO 2018). This discrepancy with the LCA outputs highlights the need to integrate the comparison between the organic rice management with other evaluations that do not use LCA indicators to obtain an all-encompassing assessment of the environmental performance of each farming system.

The main weakness of the LCA studies, which often lead to misinterpretations of its outcomes, is due to that LCA is focused on the impacts but neglects the environmental benefits and thus many aspects relevant for the long-term sustainability assessment (Notarnicola et al., 2017), such as the effect of some practices on soil physical and biological fertility, water retention and erosion, biodiversity and habitats conservation, and further ecosystems services (e.g. pest natural enemies, pollinators, landscape, resilience of food production systems, use of inputs internal to the farm).

3.2 The overall performance of organic rice farming

The assessed environmental performance of organic rice production took into account two productive levels recorded for the study area by Orlando et al. (2020), 3.91 t/ha (Q2) and 5.65 t/ha (Q3), and thus a range of yield variability consistent with the data shown in literature by similar studies (e.g. 3.83 t/ha, Hokazono and Hayashi, 2012; 4.4 t/ha, Blengini and Busto, 2009; 4.5 t/ha, Bacenetti et al., 2016; 5.3 t/ha and 6.0 t/ha, He et al., 2018). Table 5 shows the percentage variation of the environmental impacts among the management strategies for each production level.

Table 5 Percentage variation of the environmental impact (EI) categories between the management strategies (i.e. maximum vs minimum value across the analyzed managements) for each productive level (Q2 = middle yield, Q3 = upper yield).

Here we show for the first time results that suggest a considerable potential of organic rice production to mitigate its impact on natural resources, depending on the chosen agricultural practices. In particular, six LCA-indicators showed a potential of reduction over 40%, shifting from the worst-performing management to the better one, with the following Q2-Q3 average values: TE and TA - 67%, ME - 65%, PM - 61%, MFRD - 58%, and CC - 42%. The remaining ones (i.e. HTc, OD, POF, FEx, FE) showed a potential mitigation potential, with values ranging between - 8% and - 22%. Similarly, Bacenetti et al. (2016), considering different options in terms of water and soil fertility management, found a potential to decrease the environmental impacts, compared to a baseline scenario, over - 40% for most of the LCA-indicators (i.e. TE, TA, PM, MFRD, CC, OD, HT, POF).

Focusing on the impact category "climate change" (CC), the values assessed across the scenarios were characterized by variability, ranging from 623 to 1365 kg CO2 eq. (i.e. minimum-maximum values, tab. 4). Comparing this range with the values shown in literature by similar studies (i.e. LCA of organic rice system "from cradle to gate"), Yodkhum et al. (2017) found a value close to our minimum (i.e. 580 kg CO2 eq.), while Hokazono and Hayashi (2012) found, in correspondence to the highest yield (i.e. 3.8 t/ha), a value close to our maximum (i.e. 1500 kg CO2 eq.). Concerning the latter study, the CC value assessed by authors is still 10% higher than our, but the rice system that they have taken into account involved continuous flooding for a total of 180 days, vs our range of 52-101 days, and this leads to a higher potential for methane emissions.

Blengini and Busto (2009) assessed for organic rice produced in the same study area of our research (i.e. Vercelli province, in Piedmont Region) a level of GHG emissions 20% more than the conventional systems, namely of 3480 kg CO2 eq., a value about 2.5 times higher than our maximum. However, the authors performed the study in 2009 when the organic rice cultivation covered only 3% of the total cultivated area of the province: it was exceptional management in its early days, rarely adopted by the farmers. Moreover, as the same authors highlighted, it resulted in a general lack of data for organic rice farming. Therefore, it is probably that the improvement in soil and water management during the last 10 years reduces the GHG emissions due to organic rice production significantly.

Another study carried out by Bacenetti et al. (2016), in the same study area of our research (i.e. Pavia province, in Lombardy Region), assessed for a baseline scenario of organic rice cultivation a level of GHG emission much higher than our maximum and close to that found by Blengini and Busto (2009) (i.e. 3270 kg CO2 eq.). However, the authors evaluated the potential of mitigation for CC LCA-indicator as equal to – 47%, reachable thanks to improvements in crop management. The resulting value, corresponding to 1736 kg CO2 eq., falls roughly within the same order of magnitude as our maximum. However, in the authors' best management scenario, the values assessed for the CC impact category were 27% higher than our maximum. Nevertheless, in all the scenarios taken into account by Bacenetti et al. (2016): i) the green manure originated from the cover crop mixture (i.e. vetch and ryegrass) is combined each year with the further distribution of organic fertilizer, in our study, instead, when leguminous species are grown during the winter, the use of an organic fertilizer product is planned only one time each three years (i.e. CC_v1, CC_v2), ii) the use of the cover crop biomass is combined with mechanical weeding trouth several passages of comb harrow, in our study, instead, the two practices are alternative, the systems based on mechanical weeding (i.e. SD, SF) exclude the cover crop cultivation, iii) the overall no flooding period seems not be considered so large such in our study where instead all the strategies involve an aeration during July, and some of them (i.e. SD, CC_v2 and CC_v1) a post sowing dry period with different extensions (fig 1), since the aeration periods play a role in the mechanical weeding, in the management of some pests (e.g. *Lissorhoptrus oryzophilus* Kuschel 1952) and aquatic weeds (e.g. *Heteranthera reniformis* Ruiz & Pav., *Heteranthera rotundifolia* (Kunth) Griseb., *Alisma plantago-aquatica* L., *Alisma lanceolatum* With., *Ammannia coccinea* Rottb., *Ammannia robusta* Heer & Regel).

Finally, the large variability of climate change impacts assessed for organic rice farming, both in this study and in literature, is due to the corresponding large variability in yield and available patterns of agricultural practices. For example, considering the CC impact category, the practices patterns affect the contribution of the primary GHG emissions sources, influencing the underlying processes: e.g. the duration of the soil aeration period and the amount of the flooded organic matter impact the anaerobic decomposition of organic matter and consequently the methane emissions that alone cover 50-61% of the total CC values (fig. 2), as well as, the quantity of green biomass or organic fertilizer and their N and P contents determine the N and P compounds release into soil, air and water (i.e. 12-28% of CC, fig. 2), and the fuel consumption associated with the mechanization of field operations affect the CO_2 emissions (414% of the CC, fig. 2).

4. Conclusion

Large variability of environmental performance tried to assess organic rice farming, as a consequence of the yield level reached and the chosen management strategy, with a mitigation potential, shifting from the worst to the best scenario, from – 67% to – 8%, depending on the LCA impact category taken into account. The LCA-indicators that showed the higher reduction potential, and thus the larger range of variability, were the follows: TE (- 67%), TA (- 67%), ME (- 65%), PM (- 61%), MFRD (- 58%), and CC (- 42%). These results agree with the wide variability of the environmental impacts assessed for organic rice by similar studies and point out the need to consider the different agronomic options available for organic systems in the comparative studies with the conventional system to avoid significant misinterpretations the outcomes. Concerning this, for example, the maximum value of the climate change impact category, assessed in the worst scenario for organic rice (i.e. lower yield level and higher impacting management strategy; 1500 kg CO2eq), resulted in being regardless lower than half of the values evaluated by other authors in comparative studies (i.e. conventional vs organic farming systems) carried out in the same study area (e.g. 3270 kg CO2 eq in Pavia province, Bacenetti et al., 2016; 3480 kg CO2 eq in Vercelli province, Blengini and Busto, 2009). The discrepancy is mainly due to the different management patterns taken into account by the different researchers. Indeed, the environmental performances in the present study are a consequence of an improvement path followed by organic farmers. The results showed that today, the farmers could reach acceptable yield values thanks to more efficient management than in the past (e.g. more extended soil aeration, less input in green or organic manure). The acknowledgement for that performances relates to the development of the farmers' know-how (Orlando et al., 2020) and to the productive improvement connected to the long-term processes which characterize the organic systems (e.g. generation of soil fertility based on biological fertility and stable humus components; lowering of weeds pressure through the gradual introduction of other crops in rotation).

The study points out the need to review the outcomes of comparative studies, conventional vs organic systems, in the light of these new results, including in the impact assessment the organic farming variability recognized from different points of view (e.g. yield, management and practices efficiency). Moreover, the different management strategies adopted for organic rice cultivation in the present study, the SD system (i.e. based on the mechanical weed control and the use of external fertilizer products), showed the best environmental performance, with significant lower impacts for 5 out 11 LCA-indicators (i.e. CC, PM, ME, TA, TE), the SF system showed overall intermediate performance.

In contrast, the "cover-crop-based" managements (i.e. CC_v1 and CC_v2) showed the worst performance, with significantly higher impacts for 5 out 11 LCA-indicators lowest impacts only for the MFRD impact category. However, concerning this, the "cover crop-based" systems showed the more considerable impact decrease potential, in correspondence of yield improvements, and involves the "good practices" (i.e. use of cover crop and leguminous species, use of input internal to the farm) that are supported by the public authorities and by the policies for the sustainability. Finally, the "good practices" penalization by the LCA outcomes points out the need to integrate this tool, suitable for the assessment of the impact, with others, suitable for the evaluation of the environmental benefits and ecosystem services, in order to obtain a reliable, systemic and integrated assessment of the sustainability of farming systems and its management systems.

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Conflicts of interest/Competing interests

The authors declare no competing interests.

Ethics approval

Not applicable

Consent to participate

Verbal informed consent was obtained from farmers before the interviews.

Consent for publication

Verbal informed consent was obtained from farmers to publish their data anonymously.

Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Conceptualization: FO, SB, JB; LCA methodology: JB, FO; Data collection: FO, EB, VV, SA; Formal analysis: FO, JB; Visualization: FO, JB, SB; Writing–original draft: FO; and Writing– review and editing: FO, JB, EB, VV, SA and SB.

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Table 1 Environmental impact (EI) categories computed in eight scenarios for organic rice cultivation, given by four management strategies (CC_v1 = green mulching with broadcast rice sowing; CC_v2 = green mulching with dry period; SD = stale seedbed in dry paddy; SF = stale seedbed in flooded paddy), each one combined with two production levels (Q2 = middle yield, 3.91 t/ha; Q3 = upper yield, 5.65 t/ha).

EI category and abbreviation		**Scenarios**		
	Unit	*n.*	*Management strategy*	*Production levels*
climate change (CC)	kg CO 2 eq.	1	CC_v1	Q2
ozone depletion (OD)	kg CFC-11 eq.	2	CC_v1	Q3
human toxicity (HTc)	CTUh			
particulate matter (PM)	kg PM2.5 eq.	3	CC_v2	Q2
photochemical ozone formation (POF)	kg NMVOC eq.	4	CC_v2	Q3
terrestrial acidification (TA)	molc H+ eq.			
terrestrial eutrophication (TE)	molc N eq.	5	SD	Q2
freshwater eutrophication (FE)	kg P eq.	6	SD	Q3
marine eutrophication (ME)	kg N eq.			
freshwater ecotoxicity (FEx)	CTUe	7	SF	Q2
mineral and fossil resource depletion (MFRD)	kg Sb eq.	8	SF	Q3

Table 2. Patterns of agricultural practices concerning the "stale seedbed in dry paddy" (SD) and the "stale seedbed in flooded paddy" (SF) strategies, both based on mechanical weed control.

Section	**Field operation**	**Period**	**Fuel** kg/ha	**Other input** product	kg/ha
SD strategy					
Tillage	levelling	every 3 years	54.5		
	field banks	every 3 years	8.6		
	ploughing (20 cm)	end April	45.5		
	harrowing (15 cm)	early May	9.9		
	spreading	end April	34.9	humoscam	450

Organic manure					
Mechanical weeding	comb harrowing n. 7 interventions (5 cm)	n. 3 in pre-sowing 10-15 May	7.2		
		n. 4 in post-sowing 16-30 May	9.6		
Sowing	row-sowing (6 cm)	15 May	5.3	rice seeds	240
Harvesting & Storage	harvest transport	October	38.4		
			30.2		
	drying		-		
Days of flooding	67				
SF strategy					
Tillage	levelling	every 3 years	54.5		
	field banks	every 3 years	8.6		
	ploughing (15 cm)	end April	40		
	harrowing (15 cm)	early May	9.9		
Organic manure	spreading	every 3 years	34.9	horn-hoof	500
Mechanical weeding	modified smoothing 2 interventions (3 cm)	pre-sowing	3.3		
		12-17 May	3.3		
Sowing	broadcast-sowing	20 May	0.9	rice seeds	240
Harvesting & Storage	harvest transport	October	38.4		
			30.2		
	drying		-		
Days of flooding	101				

Table 3 Patterns of agricultural practices concerning the "green mulching with broadcast rice sowing" (CC_v1) and the "green mulching with dry period" (CC_v2) strategies, both based on weed control through the use of cover crops and their biomass.

Section	**Field operation**	**Period**	**Fuel** kg/ha	**Other input** product	kg/ha
CC_v1 strategy					
Tillage	levelling field	every 3 years	54.5		
	banks	every 3 years	8.6		
	ploughing (25 cm)	end August	51.4		
Cover crop	row-sowing (3 cm) combined with harrowing (15 cm)	early September	0.9	Vetch	25
				Italian ryegrass	25
	biomass chopping	15 May	9.0		

Organic manure	spreading	every 3 years	34.9	horn-hoof	500
Sowing	broadcast-sowing	14 May	0.9	rice seeds	240
Harvesting & Storage	harvest	October	38.4		
	transport		30.2		
	drying		-		
Days of flooding	79				
CC_v2 strategy					
Tillage	levelling field	every 3 years	54.5		
	banks	every 3 years	8.6		
	ploughing (25 cm)	end August	51.4		
	harrowing (15 cm)	early September	9.9		
Cover crop	broadcast-sowing	early September	7.4	Vetch	25
				Italian ryegrass	25
	biomass chopping	12 May	9.0		
Organic manure	spreading	every 3 years	34.9	horn-hoof mixture	500
Sowing	row-sowing (3 cm)	18 April	4.5	rice seeds	240
Harvesting & Storage	harvest	October	38.4		
	transport		30.2		
	drying		-		
Days of flooding	52				

Table 4 Environmental impact (EI) categories referred to 1 t of paddy rice at commercial moisture, evaluated in eight scenarios for organic rice cultivation. Legend: Δ = absolute difference Q3-Q2; CC_v1 = green mulching with broadcast rice sowing; CC_v2 = green mulching with dry period; SD = stale seedbed in dry paddy; SF = stale seedbed in flooded paddy; Q2 = middle yield; Q3 = upper yield.

EI	*Unit*	CC_v1			CC_v2			SD			SF		
		Q2	Q3	Δ	Q2	Q3	Δ	Q2	Q3	Δ	Q2	Q3	Δ
CC	kg CO_2 eq	1365	1038	-327	1296	975	-321	780	623	-157	1050	832	-218
OD	kg CFC11 eq.	0.0000387	0.0000323	-0.0000064	0.000032	0.0000256	-0.0000064	0.0000383	0.00003	-0.0000083	0.0000313	0.0000251	-0.0000062
HTc	CTUh	0.0000159	0.0000133	-0.0000026	0.0000131	0.0000105	-0.0000026	0.0000163	0.0000127	-0.0000036	0.0000128	0.0000103	-0.0000025
P M	kg PM2.5 eq.	1.18	0.89	-0.29	1.17	0.88	-0.29	0.44	0.38	-0.06	0.62	0.5	-0.12
POF	kg NMVOC eq.	2.94	2.18	-0.76	2.82	2.07	-0.75	2.7	1.99	-0.71	2.38	1.75	-0.63
TA	mol c H+ eq.	50.14	37.95	-12.19	49.97	37.79	-12.18	15.34	13.83	-1.51	24.62	20.21	-4.41
TE	Mol c N eq.	224.2	169.4	-54.8	223.9	169.1	-54.8	68.2	61.4	-6.8	109.9	90.1	-19.8
FE	kg P eq.	0.16	0.12	-0.04	0.149	0.109	-0.04	0.15	0.11	-0.04	0.147	0.108	-0.039
ME	kg N eq.	22.65	17.09	-5.56	22.62	17.07	-5.55	7.36	6.49	-0.87	11.47	9.31	-2.16
FEx	CTUe	1449	1169	-280	1245	967	-278	1483	1134	-349	1232	958	-274
MFRD	Kg Sb eq,	0.00618	0.00459	-0.00159	0.00578	0.0042	-0.00158	0.01389	0.00982	-0.00407	0.00747	0.00536	-0.00211

Table 5 Percentage variation of the environmental impact (EI) categories between the management strategies (i.e. maximum vs minimum value across the analyzed managements) for each productive level (Q2 = middle yield, Q3 = upper yield).

EI	% Variation	
	Q2	Q3
CC	-43	-40
OD	-19	-22
HTc	-21	-23
PM	-63	-58
POF	-19	-19
TA	-69	-64
TE	-70	-64
FE	-8	-10
ME	-68	-62
FEx	-17	-18
MFRD	-58	-57

line figure Click here to access/download; line figure; Figure_Orlando et al.090721.docx

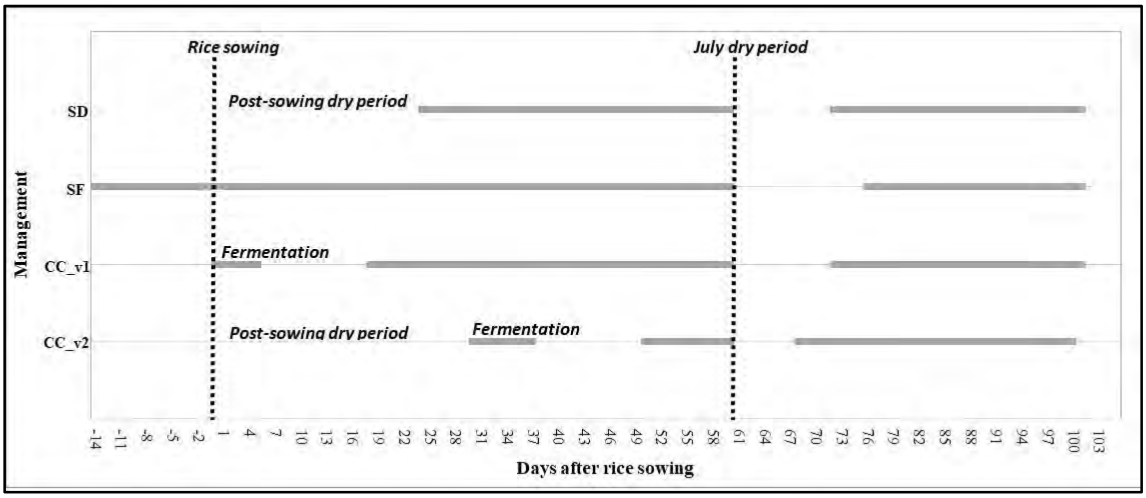

Figure 2
Irrigation schedule describing the cycles of flooding for the management scenarios, 4 characterized by a rice row-sowing and post-sowing dry period (i.e. SD and CC_v2) or rice 5 broadcast-sowing and subsequent flooding (i.e. SF and CC_v1).

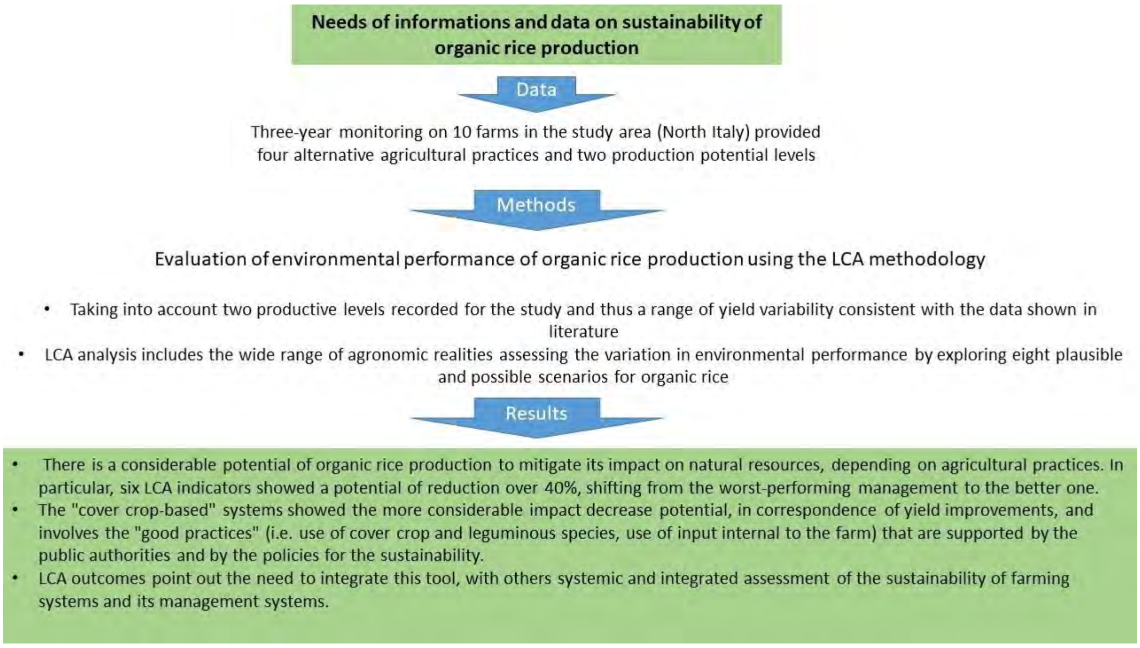

Figure 1 highlights the topic and result of the article briefly. **Figure with colour**

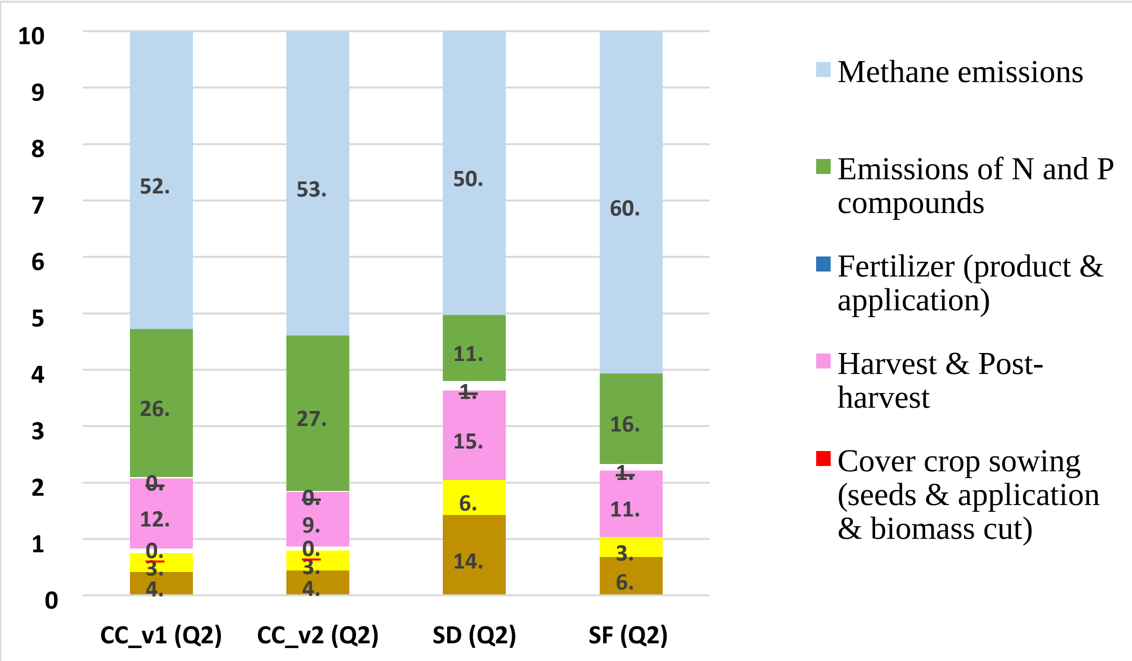

Figure 3 Percentage contribution of different sources of GHG emissions to climate change total value assessed for each management strategy (CC_v1 = green mulching with broadcast rice sowings; CC_v2 = green mulching with dry period; SD = stale seedbed in dry paddy; SF = stale seedbed in flooded paddy). **Figure with colour**

General conclusion

The environmental impacts of organic farming have been discussed controversially in the scientific community for many years. There are still conflicting views on how far organic farming can help address environmental and resource challenges and if its promotion is an appropriate policy, such as the approach to the solution of existing socio-ecological problems (Kirchmann, 2019). To date, no clear perspective on these questions has been established.

Furthermore, according to the recent research of Debuschewitz and Sanders, 2022, two main trends appear in the scientific panorama. The first concerns on the thematic framework, which compares organic farming with the other most widely used systems for which the aspects of food safety are measured by taking into account the assessment of environmental aspects that is a concept that needs a systemic vision that includes all the ecosystem services for the assessment of sustainability in agriculture. Secondly, the spatial framework creates debate as to the reference unit of measurement for evaluating net or possible environmental impacts, causing possible difficulties and dispersion of the real effects of the study due to lower yield levels typical of organic agriculture. The major problem of the contrast between cultivation methods arises if the initial question is binary (e.g. is organic farming superior to conventional agriculture, or vice versa), not considering that the step forward made from one method could give rise to a better perspective for the other.

The research carried out in this thesis emerges (Chapter I) that there is a lack of knowledge of techniques and practices for organic cultivation, especially focusing on rice cultivation in Europe. Therefore, before being able to affirm what is better or worse scientifically, it is noted that in the scientific landscape, both basic and applied research would still be necessary in order to guarantee farmers both basic materials (e.g. seeds, cultivars) suitable for the needs of organic farming and evidence and useful information to support cultivation methods. Thanks to this work, a participated method is published to study organic farming agrotechnics and their productive scenarios for the first time in Italy.

Chapter II of the thesis demonstrates the laboratory verification of what appear to be promising agricultural practices that could help in the fight against weeds. It opens to new studies about allelopathy, also considering rice allelopathic cultivars.

As reported in the introduction of each scientific research conducted, there is a lack of knowledge concerning the best practices for low-input or organic cropping systems of rice farming. These works presented assume that the causes underlying this lack are the high level of complexity and variability sources that characterise the agroecosystem of organic flooded paddy and the consequent difficulty in validating suitable agronomic solutions with the traditional research approaches show low repeatability in the organic farm reality.

Moreover, in organic cultivation, the farmers have a role in terms of professionalism and skills in adopting adaptive management, which should be improved to face uncertainty in terms of know-how-use of inputs-seed-banks.

Chapter III, starting from the management and productive scenarios presented in Chapter I, focuses on the LCA of environmental impacts of different management and productive scenarios for organic rice in Italy, underling that the "cover crop-based" systems showed the decreased potential of impact and it involves good practices supported by the CAP. On the other hand, the LCA outcomes point out the need to integrate this tool with others to evaluate the environmental benefits and ecosystem services, as is underlined in van der Werf et al 2020 because LCA methods are inadequate to consider agroecological principles designed to strengthen the resilience of food systems.

This thesis raised further scientific questions that are still in progress. The study still ongoing are regarding the following questions.

1) The environmental impacts of organic rice farming compared to conventional rice cultivation, what differences do they have in terms of GHG emissions also evaluating the biodiversity of microbial communities associated with different rice varieties?
2) What results could arise when using LCA methods associated with more systemic tools such as Sustainability Assessment of Food and Agriculture systems SAFA (FAO, 2014)? What results could arise?
3) Have the agronomic techniques and agri-environmental conditions of organic and conventional cultivation impacted the structural properties of the rice grain and the cooking properties of the main varieties suitable for the preparation of "risotto"?

The studies presented in the previous chapters made it possible to add new information to the research referring to the importance and contribution of participatory research to learn about the techniques adopted in Italy for the cultivation of organic rice. The laboratory study deepened the knowledge about the allelopathic effect of the main cover crop used by the green mulching technique, and field studies in this regard are being heightened. Chapter III takes up the production scenarios and the main methods presented in Chapter I to evaluate environmental sustainability through a widely used methodology in researching and assessing ecological impacts.

The main results presented in the chapters are disseminated through an informative project, written with the Tutor supervision and started in 2020 in collaboration with the network of researchers, professionals and farmers involved. EAFRD finances the project - Rural Development Program 2014-2020, MEASURE 1 - "Transfer of knowledge and information actions" SUB-MEASURE 1.2

- "Support for demonstration activities and information actions" OPERATION 1.2.01 "Demonstration projects and information actions" MEASURE / ACTION PSL E 1.2.01 - "Organic farming".

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