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**COMPARISON AND EVALUATION OF RICE (*Oryza sativa* L.) CROPPING
SYSTEMS FOR SUSTAINABLE AGRICULTURE**

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Abstract

In recent decades, the global agrifood system has increasingly faced variety of challenges, including climate change, soil degradation, loss of biodiversity, economic instability and the growing demand for food driven by population growth. Traditional agricultural practices adopted in rice (*Oryza sativa* L.) production, often characterised by intensive monoculture, heavy reliance on chemical inputs, and extensive resource use, have significantly contributed to these problems. The Paris Agreement aims to limit the global temperature increase to below 2°C above preindustrial levels, necessitating rapid decarbonisation across all economic sectors. The global food system alone accounts for nearly one-third of total global greenhouse gas (GHG) emissions and poses a risk to achieving the Paris Agreement's targets (Crippa et al., 2021). In the European Union (EU), agriculture contributes 12 per cent of the total GHG emissions. It is a major driver of environmental issues such as biodiversity loss and eutrophication (Leip et al., 2015).

In reply, the EU has introduced the European Green Deal (European Commission, 2019), a policy initiative that includes the Farm-to-Fork and Biodiversity strategies. Its key targets include achieving net-zero GHG emissions by 2050, reducing fertiliser inputs by 20 per cent, shifting 25 per cent of farmland to organic management, and planting three billion trees by 2030. However, the specific policy measures remain undefined and are left to individual Member States. Achieving these objectives requires a fundamental transformation in the production and consumption of agricultural goods.

Agroecology (AE) represents an integrated approach that includes ecological and social principles into the design and management of food and agricultural systems (Barrios et al., 2020). Recognized as a potential practice to meet these goals under the common agricultural policy (CAP), AE is seen by scientists as a promising approach to addressing these challenges (Peeters et al., 2021). Local examples have demonstrated that AE can enhance various ecosystem services (Boeraeve et al., 2020). However, there is a lack of comprehensive evaluation to determine whether the outcomes observed at local levels are scalable. Moreover, research has yet to assess whether scaling AE to the territorial (i.e., EU) level might introduce new challenges, such as those arising from absolute thresholds in land use or nutrient availability from biological source.

This thesis aims to apply the concepts of agroecology and sustainability to rice farming at various levels: the bibliographic level to determine what exists in the literature, the field level to test what was found in the bibliography, and finally, the farm level to evaluate the farming system in terms of sustainability and agroecology. For this, different research methods were used to retrieve data, information, and knowledge

and to arrive at an integrated assessment of rice farming activities with the tools that the scientific field provides.

All the chapters of the thesis are strictly connected. In fact, from the systematic review work, "organic" is the most recurring keyword in CO₂ mitigation strategies. Additionally, among the techniques to be adopted for mitigation, "organic fertilisation" is among those that receive the highest scores. These very important results helped decide on the field trials. Specifically, for organic fertilisation, a consortium of nitrogen-fixing biostimulant bacteria was tested for two years as a more sustainable addition to traditional mineral fertilizations. The second field study concerns the world of organic rice, with seed replication and an in-depth review of allelopathic rice varieties, which, due to their potential ability to compete with significant weeds, could be a more sustainable varietal choice in the future. In the final chapter of this thesis, after analysing the sustainability of rice cultivation both in the literature and in the field, the study focuses on the farm level. This involved a multidisciplinary survey of four rice farms (two organic and two conventional), analysed through three different studies: Life Cycle Assessment - LCA (environmental impact study), OASIS (agroecological level study), and Gross Margin (economic study).

CHAPTER 1 GENERAL INTRODUCTION

1.1 Importance of Sustainable Rice Production

Rice production and consumption exemplify agriculture's multifunctional and multiple-scale nature, providing valuable insights into sustainability transitions worldwide. Rice is cultivated in diverse ecosystems-such as upland, lowland and inland valleys- under varying climatic conditions (tropical and subtropical), and different water regimes, including lowland, upland, floating, irrigated, partially irrigated, employing both high-input and low-input methods (Zeigler and Barclay 2008, Dawe et al. 2010, Pandey et al. 2010). In many cultures, rice is synonymous with food, for example, in ancient Asian traditions, a meal without rice was not considered a meal (Anderson 1988). Confucius highlighted its centrality: *"Coarse rice for food, water to drink and the bent arm for a pillow— happiness may be enjoyed even in these"* (Confucius 1809). The cultural and spiritual importance of rice is well-documented (Anderson 1988, Hamilton and Ammayao 2003). For centuries, Mekong Delta farmers have referred to rice as 'white gold', emphasizing its role in their wealth and well-being (Cramb 2020). Beyond Asia, rice is traditionally cultivated in parts of Africa, Northern Italy and North and South America (Pandey et al. 2010; Muthayya et al. 2014; van Oort et al. 2015; Van Ittersum et al. 2016).

Global rice consumption has risen in absolute terms and in per capita, ranking from 5 kg to 250 kg annually per person, with variations across countries (FAOSTAT 2022). This increase stems from population growth in Asia, Latin America, and Africa, as well as changes in diets in Europe, Australia, and North America (Fairhurst and Dobermann 2002). As a result, rice has become a staple food. Beyond sustenance, rice fields offer diverse ecosystem services, including cultural identity, eco-tourism, climate regulation, flood-water control and water purification (Natuhara 2013; Settele et al. 2019). Modern rice production is resource-intensive, requiring land (Maclean et al. 2013) and consuming up to a quarter of the global agricultural freshwater for irrigation (Chapagain and Hoekstra 2011). However, paddy rice cultivation contributes approximately 10% of global agricultural GHG emissions (Tubiello et al. 2013). Concurrently, climate change poses significant threats to rice production through water scarcity, floods and sea level rise (Wassmann et al. 2009, Hatfield et al. 2011, Singh et al. 2017), endangering the livelihoods of millions of smallholder farmers with limited adaptive capacity (Misra 2017, Nyadzi et al. 2019, Ojo and Baiyegunhi 2020, Ho et al. 2022).

Rice also holds significance as a locally and internationally locally and internationally (Muthayya et al. 2014). The Green Revolution enormously boosted rice yields, enabling many nations to meet domestic needs and trade surpluses internationally (Borlaug 2007). However, this model has revealed its limitations.

Farmers' reliance on technology suppliers, capital, and external expertise has reduced their autonomy and decision-making creativity. Furthermore, the consolidation of power in machinery, seeds, chemicals, and distribution industries has limited their control over essential resources. Sustainability challenges, highlighted in reports like the *Club of Rome* (1972), the Brundtland Report (1987), and the Rio Declaration (1992), introduced complexity to intensive agricultural systems, particularly in developed countries. European agriculture, for instance, now contends with the risks and uncertainties that influence both learning and practice.

In different high-income countries, rice exports serve as a critical income source for farmers and a way for governments to earn foreign currency and boost their economies. However, high per-capita consumption in major rice-producing nations and the concentration of exports among a few countries contribute to market volatility (Seck et al. 2012). This volatility has led to food crises, riots, and panic during shortages (Zeigler and Barclay 2008, Dawe and Slayton 2012, Berazneva and Lee 2013), underscoring rice's importance for global food security (Seck et al. 2012, Brooks and Place 2019). Future food security is further jeopardized by projected increases in global rice demand and environmental changes (Wassmann et al. 2009, Timmer et al. 2010, Samal et al. 2022). Addressing these challenges requires consideration of rice's biophysical aspects alongside its social, economic, institutional, and cultural dimensions across production, distribution, and consumption systems.

1.2 Nitrogen fixing biostimulants

Climate change, pesticide pollution, and the growing demand for safe food production necessitate advanced, sustainable crop management techniques, including improving plant-microorganism associations. Microbial communities, classified by immense diversity, interdependence, and multifunctionality, play a critical role in regulating essential ecosystem processes. Despite their importance, knowledge of soil microbial communities and their interactions with plants remains confined. These communities control key ecological functions such as nitrogen fixation (N-fixation), nutrient acquisition, agro-waste management, biodegradation, carbon sequestration, land reclamation, bioremediation, plant growth promotion, pathogen suppression, induced systemic resistance, tolerance to stresses, and soil stability.

A plant biostimulant refers to a material containing substances or microorganisms that stimulate natural processes in plants or the rhizosphere, enhancing nutrient uptake, nutrient efficiency, tolerance to abiotic stresses, and crop quality, independent of the material's nutrient content (Ricci et al. 2019). Biological nitrogen fixation (BNF) occurs through two main pathways: symbiotic nitrogen fixation (SNF) and

asymbiotic nitrogen fixation (ANF). The symbiotic association between legume roots and N₂-fixing rhizobia, discovered in the 19th century, has been vastly studied (Lindstrom and Mousavi 2020, Brown et al. 2020). ANF, performed by free-living diazotrophs, is widespread but less efficient than SNF. Nonetheless, ANF accounts for at least a third of global BNF and is a major nitrogen source in tropical and subtropical forests (Reis et al. 2020, Sullivan et al. 2014).

Plant leaves, covering approximately 10⁹ km² and housing 10²⁶ bacterial cells, represent one of Earth's largest microbial habitats (Vorholt 2012, Zhu et al. 2022). The phyllosphere, or the surface of plant leaves, hosts diverse and abundant microbial communities (Vacher et al. 2016), which contribute significantly to plant development and health. Notably, these microbial communities act as a critical interface for colonization by free-living diazotrophic bacteria. Epiphytic and endophytic diazotrophs within the phyllosphere form close associations with plant hosts, contributing to nitrogen supply and promoting growth (Madhaiyan et al. 2015, Van Deynze et al. 2018).

Recent research has identified N₂-fixers and nitrogenase activity across diverse habitats and plant types, including the xylem sap and epiphytic phyllosphere in maize (Zhang et al. 2022, Abadi et al. 2021), the leaf endosphere of pioneer plants such as *Salix rehderiana*, *Salix ernestii*, and *Populus purdomii* (Sun et al. 2022), forest system leaves (Moreira et al. 2021, Stanton et al. 2019), and mosses in high-latitude ecosystems (Holland-Moritz et al. 2021). These phyllosphere-associated N₂-fixers support host health, enhance productivity, and contribute to global nitrogen cycling. Investigating BNF in the phyllosphere and identifying keystone N₂-fixers is crucial for understanding and managing global nitrogen cycling (Puri et al. 2016).

Although BNF occurs across various ecosystems and plant hosts, limited studies focus on the contribution of N₂-fixers to global nitrogen cycling in agricultural contexts. Achieving a sustainable food supply with minimal environmental impact demands more attention to BNF rates in agricultural ecosystems. Understanding the ecological adaptations of N₂-fixers and addressing constraints to BNF in the phyllosphere is vital to leveraging plant-associated diazotrophs for biologically fixed nitrogen in agricultural production. Evidence suggests that phyllosphere microhabitats host diverse N₂-fixers in ecosystems such as grasslands, agricultural lands, forests, and high-latitude regions, highlighting their potential for broader application in sustainable agriculture.

1.3 Allelopathic rice

Rice, playing a role of significant importance for agriculture, has been studied for a long time to obtain its allelopathic traits, which are found to be present in its extracts, roots exudates and decomposing residues (Kato-Noguchi et al., 2013); these chemical compounds, in addition, are also released into the

environment through volatilization and make this cereal one of the most suitable crops for the use of this phenomenon (Jensen et al., 2008).

Research has shown that approximately 4% of rice varieties on Earth have allelopathic potential against some of the most harmful weeds, such as *Echinochloa crus-galli*, *Cyperus difformis* and *Heteranthera limosa* (Alam et al., 2015).

In addition, rice variety and origin may regulate allelopathic behaviour, for example cultivars belonging to the japonica subspecies exhibited greater weed suppression than Indica-type varieties and hybrids between these two types (Khanh et al. 2007). Many of these compounds released into the soil are secondary metabolites and include alkaloids, phenols, flavonoids, terpenes, and glucosinolates, which do not participate strongly in the primary metabolism of the plant but are essential for its survival. Cytokines, fatty acids, indoles, momilactones, phenolic acids, steroids, and other constituents are also added to these. (Khanh et al., 2007). Plants mainly synthesize these compounds during their growth and development; for this reason, allelopathy is more critical as the season progresses, when the crop is more mature and has a higher density on the worked surface (Lin et al., 2010). Furthermore, it has been shown that environmental stresses could increase the allelopathic strength of some plants.

However, the rice cultivars that possess these characteristics currently available on the market are limited in number and their yield and the quality of final product do not meet the needs and standards of the rice industry. Therefore, the development of commercially acceptable varieties is highly desired, so this solution is increasingly being studied (Alam et al. 2015).

Moreover, to obtain agriculture based on a low input of products introduced into the ecosystem, the use of allelopathy must be supported by other techniques which limit the development of unwanted species, such as bioherbicides and tillage methods, requiring careful integration (Masum et al., 2015).

Rice allelopathy is not only determined by a plant-to-plant interaction but also involves soil-mediated chemical interventions, which can be regulated by various environmental factors. Hence, these effects are key methods to improve its efficacy. Studies have shown that habitat changes could influence the synthesis and type of allelochemicals released (Gaofeng et al., 2018).

Allelopathy also offers an attractive natural option for decreasing nitrification and improving the efficiency of nitrogen use in agricultural systems. This process can occur by incorporating crop residues into the soil, with plant roots releasing allelochemicals that inhibit the activity of several vital enzymes.

Allelochemicals can be hydrophilic or hydrophobic, are absorbed by the soil surface, coat plant residues and are then carried away by the wind. Once released, they spread through the soil and can be carried by

water; they are also found in the rhizosphere and have been shown to interact with organisms through root contact (Amb et al., 2016).

Several investigations have resulted in the detection of rice allelochemicals in the form of phenolic compounds and momilactones. In addition, over 200 different compounds have been reported in rice root exudates and subsequently placed into three distinct classes, phenols, phenyl alkanoic acids and indoles, and further classes of secondary metabolites determined by radical exudates such as phenols, alkyl resorcinols, momilactones B, carbohydrates, amino acids and flavones.

This last has been shown to play a decisive role in the interaction between rice and other organisms present in the rhizosphere and possess a high inhibitory action compared with other substances, such as phenols (Amb et al., 2016).

The sites of action of allelochemicals hold a significant charge during the culture development process, as they assist in determining the increase of the desired chemical to achieve its most optimal release (Chung et al., 2018).

Most of the rice cultivars that can be used to exploit their allelopathic traits have the availability of more than one chemical compound to be released into the soil to inhibit the growth of weeds. Allelopathy has also shown significant implications in the biological control of pathogens to manage possible diseases to which plants are susceptible. Several studies, in fact, have successfully demonstrated optimal disease control through the use of both rotations between allelopathic cultivars and allelochemicals in pure/raw form. The presence of allelochemicals in rice, such as phenolic acids and momilactone A, makes this cereal a stronger competitor against insect pests and other pathogens (Kato-Noguchi et al., 2013).

The application of allelopathy through genetic manipulation, using molecular genetics and biotechnology, can be considered a successful tool in the defence of rice cultivation for the control of weeds, insects and pathogens. For this reason, it is essential to recognize the genes responsible for potential allelopathy and also to understand the structure and genetic control of the allelopathic trait to incorporate it into the development and growth programs of the crop itself (Patni et al., 2018).

It has been confirmed that allelopathy in rice is a genetic trait that can be induced and associated with the molecular regulation of secondary metabolic pathways. Twenty-four genes have been classified, and divided into 5 groups, according to the site of action on which they act: primary metabolism, synthesis of allelochemicals in the form of phenols, regulation of plant growth/cell cycle, transduction of responses/signals due to stress and synthesis/degradation of proteins according to their functions (Song et al., 2008).

A crucial aspect of allelopathy is its effect on the nutritional value of foods and crops subject to this phenomenon. A change in the combinations or levels of secondary metabolites in crops does not affect the quality of food products (Sitthinoi et al., 2013). The structure of allelochemicals can be used as a model for the synthesis of new pesticides that are less harmful to the environment than similar chemical products.

1.4 Original Agroecological Survey and Assessment System

OASIS ("Original Agroecological Survey and Assessment System") is among the first, or possibly the very first, analysis framework specifically designed to evaluate agroecological systems at a national level.

OASIS initially emerged as part of a joint project between FAO and Agroecology Europe, aimed at promoting agroecology in Europe and Central Asia by mapping agroecological farming practices. Its broader goal is to assess a country's agroecology state (Škorjanc K, 2020). Evaluating the agroecological status is a crucial first step for determining how to effectively communicate and disseminate the principles and practices of agroecology. For example, farms identified as agroecological and performing well could serve as demonstration farms, thus encouraging further transition toward agroecology.

OASIS represents what we might call a "paradigm shift." Unlike earlier tools (e.g., SAFA, FAO), its goal is not to evaluate the sustainability level of a farm, but rather to determine where it stands within an agroecological transition (Škorjanc K, 2020).

Being a recently introduced tool, OASIS incorporates the lessons learned from previous tools, adapting them to current needs and opportunities. Maintaining an integrated and multidisciplinary approach to all aspects of sustainability (social, environmental, and economic), it has expanded and structured these dimensions to address existing gaps, especially in socio-political and economic indicators. Moreover, OASIS has among its strengths being versatile, intuitive, and farmer-oriented. It considers the farmer's perspective while also providing practical information and impact indicators that can be used by policymakers and researchers alike.

Another important and innovative aspect of OASIS is that its agroecological indicators assess the implementation of values, practices, and processes rather than specific outputs. The premise is that when a farmer correctly applies practices tested and shown to align with agroecological theory, they will also benefit from all the multidimensional services agroecology provides.

One of the main limitations, related to this last point, is that agroecology is a developing science, and there are still many knowledge and technical gaps to fill. For this reason, OASIS is currently being tested

in various European countries, with ongoing adjustments to suit both the context in which it is tested and the advancements in agroecology as a science.

Data is collected through an interview with the farmer about the practices implemented on the farm and an on-site visit to the farm itself. The evaluator is expected to have a solid understanding of agroecology in general and, specifically, of the region where the assessment occurs, as technologies, techniques, and best practices will vary significantly from one environment to another.

Most indicators are evaluated semi-quantitatively on a scale from 1 to 5, with the maximum score representing the benchmark and corresponding to the highest level of agroecological practice or system.

The indicators are grouped into five main pillars (Fig. 1.1):

- Adoption of agroecological practices
- Economic aspects
- Socio-political aspects
- Farm's environmental and biodiversity impact
- Resilience (both climatic and economic)

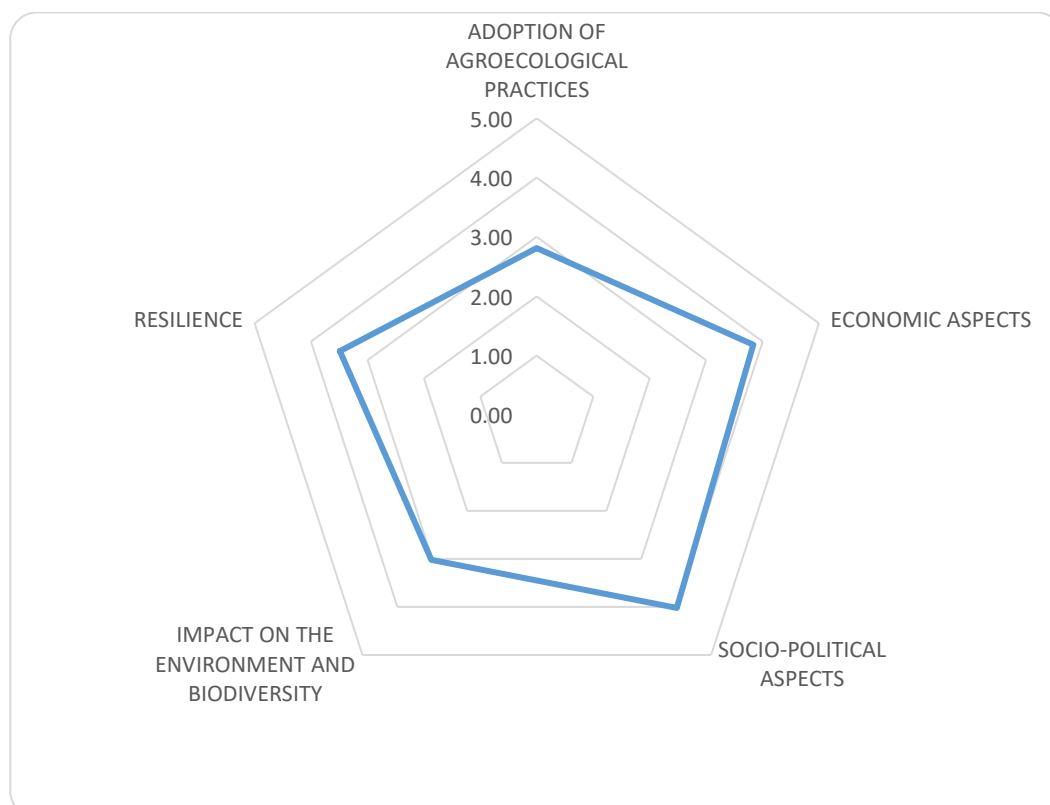


Figure 1.1: OASIS indicators

1.5 LCA Life Cycle Assessment

In recent years, energy-related issues have increased significantly, focusing on the rational use of resources, the environmental effects of pollutant emissions, and the depletion of non-renewable resources (Stoppato A., 2008). This shift is driven by the need to better understand the intricate relationship between environmental sustainability and socio-economic development. The growing demand for services and infrastructure today, while advancing progress and enhancing the quality of life, often generates environmental challenges, posing difficulties for current and future generations (United Nations, 2018).

Given these complexities, a widespread consensus exists on the urgency of adopting sustainable practices (Walker et al., 2018). A critical aspect of this transition is the deployment of practical tools for environmental impact assessment, with Life Cycle Assessment (LCA) emerging as a key instrument (Hauschild et al., 2018; Varun et al., 2009; Asdrubali et al., 2015). LCA provides a comprehensive evaluation of the environmental consequences of products and services throughout their life cycles, encompassing stages from production to disposal. This approach is often called "cradle to grave" (Cheng et al., 2022; Ludin et al., 2018) as it examines all stages, including raw material extraction, manufacturing, use, and final waste disposal.

LCA has expanded rapidly, particularly as a comparative tool for assessing the environmental footprints of different products or services (Jungbluth, 2005). It aids in selecting environmentally preferable alternatives, supports green design principles, and identifies potentially harmful impacts (Hellweg et al., 2014). Over time, LCA has transitioned from an essential environmental assessment tool to a comprehensive instrument for sustainability studies, influencing decisions ranging from local operational choices to broad strategic policies. Initially focused on direct impacts such as waste generation and energy consumption, LCA now addresses broader environmental issues, including acidification, human toxicity, ozone layer depletion, resource depletion, and water use (ISO 14040, 2006).

The LCA process follows a systematic structure, offering a holistic framework for environmental decision-making. According to ISO standards 14040:2006 and 14044:2006, it comprises four phases (de Saxcè et al., 2012):

1. **Goal and Scope Definition:** This foundational phase outlines the objectives, system boundaries, and functional unit of analysis. System boundaries determine which processes are included, while the functional unit enables consistent comparisons of products or systems.

2. Life Cycle Inventory (LCI): This phase involves data collection on material and energy flows based on the defined boundaries. The resulting inventory forms the basis for subsequent impact assessments.
3. Life Cycle Impact Assessment (LCIA): This phase comprises several sub-steps:
 - Impact Assessment: Calculation of environmental impacts using LCI data.
 - Classification: Grouping data into distinct impact categories.
 - Characterization: Quantifying the relative contributions of materials/emissions within each category.
 - Standardization and Valuation: Allow for detailed impact analysis, though valuation is more subjective than standardization.
4. Interpretation and Recommendations: Results are interpreted to identify improvement opportunities and recommend actions.

The successful application of LCA depends on sophisticated software tools and extensive databases. Leading tools include:

- OpenLCA: An open-source LCA tool developed by GreenDelta in 2006, valued for its accessibility and comprehensive analysis capabilities (Green Delta OpenLCA, 2021).
- BEES (Building for Environmental and Economic Sustainability): A tool by the National Institute of Standards and Technology (NIST), integrating environmental and cost performance assessments (NIST BEES, 2021).
- GaBi: Offers robust LCA modeling and reporting supported by extensive databases (Sima Pro, 2021).
- SimaPro: A well-established tool in industry and academia, recognized for its transparency and robust analytical capabilities (ifu Hamburg, 2024).
- Umberto: Known for its flexibility, it supports complex LCA modelling and data integration (Millet et al., 2007).

The reliability of LCA results hinges on the quality and origin of baseline data (Hollerud et al., 2017; Miah et al., 2017). Early in the process, obtaining tailored primary data from key suppliers is crucial, especially given this stage's scarcity of detailed information. While public and private databases are valuable (European Commission, 2013), assessing their representativeness relative to the studied system is

essential. Method differences and the lack of integration strategies remain significant challenges (Lehmann et al., 2016).

To enhance comparability, the European Commission introduced the Product Environmental Footprint (PEF) method via Recommendation 2013/179/EU (Bach et al., 2018). PEF aims to create standardized principles for evaluating environmental performance across product life cycles (Manfredi et al., 2015). It includes 16 defined impact categories and ensures consistency by normalizing environmental impacts across life cycle stages. However, its rigid methodological specifications reduce flexibility compared to ISO standards (Pyay et al., 2019). From 2013 to 2016, the European Commission conducted 26 pilot projects across diverse sectors (Corradini et al., 2019), demonstrating the PEF's potential to improve product sustainability. Since then, interest in PEF has grown, reflecting its capacity to align product design and consumer behavior with sustainability goals (Kuo et al., 2019; Wu et al., 2021).

This increasing adoption underscores the vital role of LCA and PEF in shaping sustainable development policies and promoting environmentally conscious decision-making globally.

CHAPTER 2 STRUCTURE OF THE THESIS

2.1 General objectives

The general objective of this study is to evaluate which agronomic solutions can be used to make rice cultivation more sustainable in terms of the agroecological approach. To achieve this objective, the study began with a literature review, then moved to field studies, and finally focused on the farm level and the available tools for its integrated evaluation and assessment

2.2 Specific objectives

- Evaluate which solutions could have a more significant impact on CO₂ mitigation.
- Evaluate the response of rice to the use of nitrogen-fixing biostimulants in terms of LAI, biomass, and yield
- Assess and characterise allelopathic rice varieties to lay the foundation for future research on the subject.
- Assess the sustainability, environmental impact, agroecological structure, and economic situation of four representative rice farms in Northern Italy.

The links between the chapters is reported in the Fig 2.1 below:

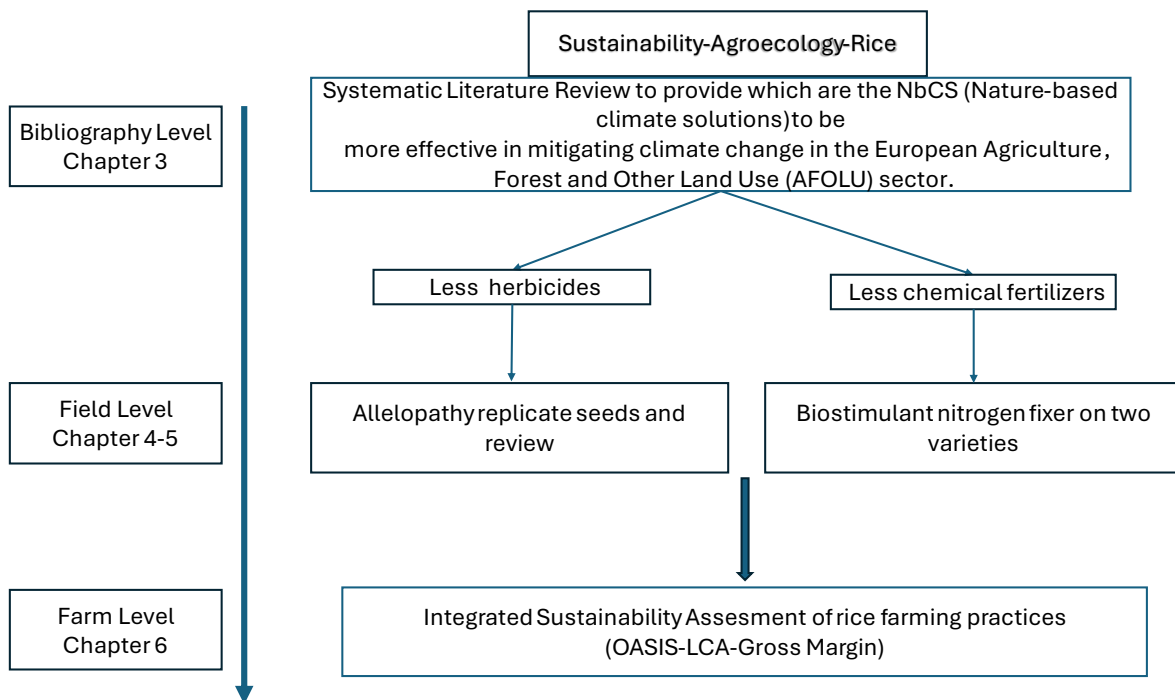


Figure 2.1: graphic scheme of the thesis

As it is presented in its current form, the PhD thesis is structured on several papers developed at different scales on integrated but distinct problems.

For this reason, some of the research has already been developed in all its forms, including that of a publication. In contrast, others require final, non-substantial, but formal work before they can be submitted to scientific journals. More specifically, chapters 3 and 6 are already in the final form of a scientific paper and have already been submitted to scientific journals. Chapters 4 and 5 contain descriptions of the research carried out on biostimulants and allelopathic varieties but still have to be formulated in such a way as to constitute a scientific paper ready to be submitted to journals. This will be done in the coming months.

CHAPTER 3 ESTIMATING MITIGATION POTENTIAL AND COST-EFFECTIVENESS OF NATURE-BASED CLIMATE SOLUTIONS IN EUROPE. A SYSTEMATIC REVIEW PROTOCOL

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Abstract

Enhancing the contribution of the Agriculture, Forestry and Other Land Use (AFOLU) sector towards climate mitigation goals is fundamental. In the European Union, the AFOLU sector heavily contributes to global GHG emissions and simultaneously presents significant mitigation potential, also through its capacity to act as a carbon sink. Several modelling tools are used to quantitatively estimate the potentials of different mitigation options at the EU scale. Estimates might strongly depend on different modelling assumptions, such as the potential uptake of each option (e.g. land extension in hectares), different scenario analyses on land share and land use change, as well as mitigation potentials per unit of uptake (e.g. per hectare) as derived from different literature sources. Sometimes large discrepancies emerge across different estimates on the same mitigation options.

Here, we propose a protocol for systematic review, with the purpose of summarising available estimates of the mitigation potential of different options within the European AFOLU sector, including their cost-effectiveness. The protocol follows the Reporting Standards for Systematic Evidence Syntheses (ROSES) and the review question follows the PICOC (population, intervention, comparison, outcome and context) framework, throughout subsequent steps: a) preliminary searches for relevant articles on Web of Science and Scopus, to better frame review questions over different mitigation options; b) definition of a comprehensive search string and inclusion criteria, to guide the screening process; c) screening of abstracts and full-text articles; d) extraction of relevant information, thanks to a standardised codebook; e) perform both a narrative and quantitative synthesis.

We also used a pilot set of 14 articles, retrieved from preliminary searches, to better define the search strings, the codebook for data extraction, the structure of the dataset and to preview the most frequently reported categories of mitigation options.

3.1 Background

NbCS are climate mitigation measures for the AFOLU sector, which involves land and ecosystems management and includes both the Agricultural and the Land Use, Land-Use Change and Forestry (LULUCF) sectors. These two sectors account for approximately 37% and 31% of global land area, respectively, and these percentages rise to 41% and 40% when considering only the European Union (FAO, 2020; Kowalczewski et al., 2021; The World Bank, 2024). The agri-food sector accounts for approximately 4.3% of global gross domestic product (GDP) and the agricultural sector alone accounts for a share of more than a quarter of global employment (International Labour Organization, 2023). The AFOLU sector is responsible for 13–24% of global greenhouse gas (GHG) emissions (Crippa et al., 2021; Nabuurs et al., 2022). AFOLU carbon dioxide (CO₂) emissions are mainly caused by the LULUCF sector and deforestation (Tubiello et al., 2021), and make up about 50% of total net emissions by the AFOLU sector (Nabuurs et al., 2022). Agriculture produces methane (CH₄) and nitrous oxide (N₂O) emissions, which are both increasing (FAO, 2021). The former are mainly caused by enteric fermentation from animal agriculture, and the latter by manure management and nitrogen fertilisers (Jia et al., 2019).

In the future, the global population is expected to rapidly grow (Godfray et al., 2010), causing food demand to double by 2050 (Tilman et al., 2011) and expanding environmental impacts related to the AFOLU sector (Kehoe et al., 2017). Mitigation is fundamental to address climate change, and it can be achieved both by decreasing the amount of emissions released into the atmosphere and by reducing the current concentration of CO₂ by enhancing sinks. AFOLU is a key sector in addressing climate change mitigation, since it is the only GHG sector offering carbon sinks (Nabuurs et al., 2022). Currently, managed ecosystems around the world absorb about 30% of anthropogenic CO₂ emissions (Nabuurs et al., 2022). The European LULUCF sector is a net carbon sink, though the rates of carbon sequestration from the atmosphere are decreasing (Kowalczewski et al., 2021). The adoption of climate mitigation measures in the AFOLU sector can provide 20–30% of global mitigation needed to meet the IPCC 1.5°C target (Nabuurs et al., 2022).

Therefore, NbCS, which include conservation, restoration and improved management strategies, are needed in order to reduce the negative impacts of the AFOLU sector and to improve its contribution to climate change mitigation (Novick et al., 2022). Examples of NbCS which can potentially contribute to this shift are forest conservation and reforestation (Houghton et al., 2012; Roe et al., 2021); agroforestry, a nature-based solution which contributes to the absorption of CO₂ from the atmosphere as well as reducing the effects of extreme weather events (Hernández-Morcillo et al., 2018; Ramachandran Nair et al., 2009); bioenergy use, which refers to the practice of producing energy from crops to offset the cutback in fossil fuels (Lemus & Lal, 2005). Other examples improved manure management (Cuéllar & Webber, 2008; Petersen et al., 2013), interventions on enteric fermentation (Hristov et al., 2015; Ross et al., 2014), energy efficiency (Schneider & Smith, 2009), land sparing (Lamb et al., 2016), cover crops (Kaye &

Quemada, 2017), no-till farming (Abdalla et al., 2013), biochar (Woolf et al., 2010). Supply-side NbCS are complemented by demand-side mitigation measures, such as plant-based diets (Hallström et al., 2015; van Dooren et al., 2014) and food loss and waste reduction (Roe et al., 2021). These strategies have been demonstrated to have an interesting potential in reducing GHG emissions (Bryngelsson et al., 2016; Scherer & Verburg, 2017).

Besides identifying which NbCS can mitigate climate change, it is equally important to estimate the mitigation potential of each of these measures, to better understand their real efficacy, and eventually encourage the design of *ad hoc* agricultural policies. At the same time, it is interesting to consider the implementation costs of NbCS and therefore analyse their cost-effectiveness. However, identifying the mitigation potential of these interventions is not easy. In addition to variability due to possible random errors in measurement and estimation methods, the uncertainty is mainly due to two factors: on the one hand, the mitigation potential of each NbCS often depends on the specific environmental and climate conditions of the region where it is adopted (Smith et al., 2013); on the other hand, there is variability due to the different methods of estimating mitigation potential that are employed in the various studies (Freibauer et al., 2004; Lal et al., 2007). Moreover, the growing literature on the subject has led to an increasing need to collect and present the knowledge acquired to date in a synthetic manner. In this sense, literature reviews can be very helpful. Recent literature reviews centred around climate mitigation, focusing on either supply-side (Smith et al., 2007), demand-side strategies (Hallström et al., 2015) or both (Scherer & Verburg, 2017). Most of these reviews identify promising mitigation strategies and their potential benefits (Freibauer et al., 2004; Lal, 2004), but focus on supply-side mitigation strategies and generally overlook demand-side strategies, despite the fact that agronomic strategies are unlikely to be effective and sufficient unless they are coupled with changes in consumer behaviour (Scherer & Verburg, 2017), and a diet shift specifically was found to have a mitigation potential up to 50% with respect to the GHG emissions related to the current diet (Hallström et al., 2015). A meta-analysis by German et al. (2017) explores the relationships between different environmental sustainability indicators, including GHG emissions, and compares estimates across the zero-tillage climate mitigation strategy and other management practices. Buma et al. (2024) explore the global mitigation potential of 43 alternative NbCS through a literature review and an expert elicitation, and find that some remain uncertain in terms of mitigation efficacy. Previous research has significantly enhanced our comprehension, yet an updated systematic literature review about the mitigation potential of different NbCS in Europe and their cost-effectiveness is valuable for several reasons. First, the systematic review methodology, in contrast to most prior reviews on the topic, allows to synthesise current scientific evidence in an unbiased and comprehensive manner, which is useful in generating a framework to effectively inform policy-making and practice (Pullin, 2012), and is increasingly employed in the agricultural sciences (Koutsos et al., 2019); also, it is particularly useful because data on mitigation potential and cost-effectiveness are usually

expressed in numerical terms, which can be collected and compared thanks to this methodology. Second, it is important to consider supply-side and demand-side mitigation strategies together (Scherer & Verburg, 2017). Third, to our knowledge there is no literature review about the mitigation potential and the cost-effectiveness of alternative NbCS modelled for European countries, which is essential to be able to provide European policymakers with tailored policy suggestions.

The aim of this systematic literature review is to provide a clear picture of which NbCS is more effective in mitigating climate change and less expensive in the AFOLU sector. A second objective is to synthesise the knowledge created by papers which model or simulate the potential mitigation contribution of a specific geographical area (a continent, a country, a region, etc.), and not only provide a unitarian estimate. The third objective is to understand the degree of scientific uncertainty that exists on these subjects. Protocols for systematic literature reviews are a requirement of the Reporting Standards for Systematic Evidence Syntheses (ROSES) (Haddaway et al., 2017). The main aim of this systematic review protocol is to ensure that the ROSES standards for systematic reviews are followed.

3.2 Protocol design

Systematic literature reviews serve the purpose of answering a pre-defined research question by synthesising evidence found in previous research. The review protocol aims at minimising bias in the review by setting out the methodology in advance and pre-specifying the eligibility criteria necessary to guide the review process (Higgins et al., 2023). The review protocol will follow the ROSES guidelines (Haddaway et al., 2017), and the checklist can be found in the Supplementary materials. We used six exploratory queries to develop the final search string, and we extracted a subset of eight articles to pilot the data extraction and coding to define the protocol design. Preliminary results from the pilot search are shown in the last section of this protocol.

3.3 Defining the research question

A well formulated research question should contain a series of elements, which can be summarised in the acronym PICOC (Population, Intervention, Comparator, Outcome, Context) (Booth et al., 2012, 2021; Petticrew & Roberts, 2008). The definitions of these elements and the application in our study are described in Table 3.1. The application of the PICOC framework allows us to formulate the following research question: what is the mitigation potential and cost-effectiveness (Outcome) of NbCS implemented in the AFOLU sector (Intervention) to enhance climate change mitigation (Population) in Europe (Context), compared to a baseline scenario without the adoption of such measures (Comparator)?

Table 3.1: Key elements of the research questions according to the PICOC framework. Definitions are based on Booth et al. (2012, 2021).

Element	Definition	Application
Population	The problem or situation dealt with by the research.	Primary studies reporting the results of models or calculations aimed at estimating the mitigation potentials of NbCS applied to the AFOLU sector of specific geographical areas over a certain period of time. Secondary studies reporting syntheses of such mitigation potential estimates.
Intervention	Existing and relevant options to address the problem.	NbCS, including demand-side measures (e.g., afforestation, agroforestry, biochar, bioenergy, cover crops, diet shift, food waste reduction, reduced tillage, etc.).
Comparator	The alternative, or control scenario with respect to the intervention.	The baseline scenario with no adoption of the intervention.
Outcome	The measure to assess the effect of the intervention in the population.	The mitigation potential, measured as Mg-CO ₂ -equivalents per year (e.g. aggregated GHGs emissions, avoided carbon stocks emissions, soil organic carbon (SOC) sequestration, changes in woody biomass carbon stocks, permanent carbon storages) and implementation costs.
Context	Particular context, setting or areas of the research.	Europe or a given area of the European continent.

3.4 Literature search strategy

The search strategy aims at selecting the relevant articles in peer-reviewed journals, with no restriction on publication year. We first explore relevant studies by running a series of pre-screening search strings on the Web of Science Core Collection, covering potential keywords and using Boolean constructs (full list in the Supplementary materials).

Second, we build the main search string to query the two largest multidisciplinary scientific databases, namely Web of Science (Clarivate)’s Core Collection and Scopus (Elsevier), and the data will be harmonised (Table 3.2). The choice of using data from two different sources significantly increases the comprehensiveness of the work, and is done following the example of similar systematic reviews carried out in our research field (Jones et al., 2023; Mouratiadou et al., 2024; Sánchez et al., 2021). The search will be launched on the articles’ title, abstract and keywords. The main search string is arranged into four sections, each defined by keywords and their synonyms. The first section contains GHGs measurement units (e.g., "carbon", "carbon equivalent", "C/year", "CO₂/yr", "CO₂eq year-1" etc.), without explicitly

mentioning either the agricultural sector or the concept of mitigation. The second section includes keywords concerning the concept of mitigation (e.g., "negative emission", "mitigation", "sequestration", "sink", "removal" etc.), while the third one refers to the agrifood system and ecosystems conservation sector (e.g., "food", "agriculture", "climate-smart practices", "AFOLU", "LULUC", "reforestation" etc.). Lastly, keywords are added referring to the European continent and countries, with the intention of restricting the search to this specific world region. We decided to carry out the systematic review on all articles reporting on mitigation potentials, prioritising the ones which also deal with economic costs.

Third, we include additional articles, which are relevant to our topic, known to co-authors or found through other channels. We export the results from all the search stages and remove duplicates based on the DOI and article title. All studies will be assigned a unique study ID. In the cases where the DOI is missing, we replace it with the text string of authors' names, in order to avoid said articles to be deleted in the merging process. The search will be updated in case the submission of the final results to a peer-reviewed journal will be made more than twelve months after the current search was performed.

Table 3.2: Final search strings launched in the Web of Science and Scopus databases

Database	Search string
Web of Science	(TS= ("Gt" OR "Gigaton*" OR "Gton*" OR "Mt" OR "Megaton*" OR "Mton*" OR "kt" OR "kiloton*" OR "kton*" OR "Pg" OR "Petagram*" OR "Pgram*" OR "Tg" OR "Teragram*" OR "Tgram*") near/1 ("CO2-eq*" OR "CO2eq*" OR "CO(2)-eq*" OR "CO(2)eq*" OR "C" OR "carbon*" OR "C equivalent*" OR "C eq*" OR "carbon equivalent*" OR "C-equivalent*" OR "C-eq*" OR "carbon-equivalent*") near/1 ("per year" OR "a year" OR "/year" OR "/yr" OR "yr-1" OR "yr(-1)" OR "year-1" OR "year(-1)" OR ((GtCO2* OR Gt-CO2* OR Gt-C* OR GtC* OR MtCO2* OR Mt-CO2* OR MtC* OR Mt-C* OR ktCO2* OR kt-CO2* OR ktC* OR kt-C* OR PgCO2* OR Pg-CO2* OR PgC* OR Pg-C* OR TgCO2* OR Tg-CO2* OR TgC* OR Tg-C*) near/1 ("per year" OR "a year" OR "/year" OR "/yr" OR "yr-1" OR "yr(-1)" OR "year-1" OR "year(-1)")))) AND TS= ("food*" OR "agricul*" OR "soil*" OR "diet*" OR "farm*" OR "food waste*" OR "livestock*" OR "afforest*" OR "conservat*" OR "land use" OR "agroforest*" OR "forest*" OR "wetland*" OR "peatland*" OR "grassland*" OR "agroecolog*" OR "agro-ecolog*" OR "crop diversification*" OR "climate-smart pract*" OR "rumen*" OR "enteric" OR "ruminant*" OR "meat*" OR "dairy" OR "arable*" OR "Restoration*" OR "woodland" OR "rangeland" OR "deforest*" OR "reforest*" OR "biochar" OR "manure" OR "fire management" OR "landscape feature*" OR "tree*" OR "grazing" OR "anaerobic digest*" OR "cropland" OR "fertilis*" OR "nitrification" OR "legume" OR "paddy" OR "tillage" OR "nitrogen fix*" OR "burn*" OR "rice" OR "sexed semen" OR "breeding" OR "afoul" OR "lulcc") AND TS= (("negative emission*" OR emission* OR carbon OR CO2 OR GHG OR methane OR "nitrous oxide" OR N2O OR CH4 OR greenhouse OR warming OR climat*) near/3 ("mitigat*" OR "stor*" OR "sequest*" OR "sink*" OR "remov*" OR "reduc*" OR "reservoir*"))

AND LANGUAGE: (English))

Scopus

(TITLE-ABS-KEY(("Gt" OR "Gigaton*" OR "Gton*" OR "Mt" OR "Megaton*" OR "Mton*" OR "kt" OR "kiloton*" OR "kton*" OR "Pg" OR "Petagram*" OR "Pgram*" OR "Tg" OR "Teragram*" OR "Tgram*") w/1 ("CO2-eq*" OR "CO2eq*" OR "CO(2)-eq*" OR "CO(2)eq*" OR "C" OR "carbon*" OR "C equivalent*" OR "C eq*" OR "carbon equivalent*" OR "C-equivalent*" OR "C-eq*" OR "carbon-equivalent*") w/1 ("per year" OR "a year" OR "/year" OR "/yr" OR "yr-1" OR "yr(-1)" OR "year-1" OR "year(-1)") OR ((GtCO2* OR Gt-CO2* OR Gt-C* OR GtC* OR MtCO2* OR Mt-CO2* OR MtC* OR Mt-C* OR ktCO2* OR kt-CO2* OR ktC* OR kt-C* OR PgCO2* OR Pg-CO2* OR PgC* OR Pg-C* OR TgCO2* OR Tg-CO2* OR TgC* OR Tg-C*) w/1 ("per year" OR "a year" OR "/year" OR "/yr" OR "yr-1" OR "yr(-1)" OR "year-1" OR "year(-1)"))))

AND TITLE-ABS-KEY("food*" OR "agricul*" OR "soil*" OR "diet*" OR "farm*" OR "food waste*" OR "livestock*" OR "afforest*" OR "conservat*" OR "land use" OR "agroforest*" OR "forest*" OR "wetland*" OR "peatland*" OR "grassland*" OR "agroecolog*" OR "agro-ecolog*" OR "crop diversification*" OR "climate-smart pract*" OR "rumen*" OR "enteric" OR "ruminant*" OR "meat*" OR "dairy" OR "arable*" OR "Restoration*" OR "woodland" OR "rangeland" OR "deforest*" OR "reforest*" OR "biochar" OR "manure" OR "fire management" OR "landscape feature*" OR "tree*" OR "grazing" OR "anaerobic digest*" OR "cropland" OR "fertilis*" OR "nitrification" OR "legume" OR "paddy" OR "tillage" OR "nitrogen fix*" OR "burn*" OR "rice" OR "sexed semen" OR "breeding" OR "AFOLU" OR "LULUC")

AND TITLE-ABS-KEY(("negative emission*" OR emission* OR carbon OR CO2 OR GHG OR methane OR "nitrous oxide" OR N2O OR CH4 OR greenhouse OR warming OR climat*) w/3 ("mitigat*" OR "stor*" OR "sequest*" OR "sink*" OR "remov*" OR "reduc*" OR "reservoir*"))

AND (LIMIT-TO (LANGUAGE, "English"))).

3.5 Screening process

The first phase of screening will consist in reading the abstracts of the selected articles. At least two researchers will read the abstracts and exclude those that were ineligible according to the inclusion criteria defined in Paragraph 6. During this phase, we expect few papers to raise doubts concerning their inclusion or exclusion in the final list. In those cases, full-text reading will be conducted to determine the ultimate choice, and the same inclusion criteria will be applied. In addition, we will exclude articles if it is impossible to find the full text. We will undertake the following process to check for consistency of decisions: we estimate that around 10% of titles and abstracts will be read by at least two reviewers, whereas at least two reviewers will go through about 5% of all the full texts read during the screening process. Should any disagreement emerge between two reviewers, this will be discussed among the review team and the final decision will be made by a this-party (Koutsos et al., 2019).

3.6 Inclusion criteria

The relevance of articles to be ultimately considered in the systematic review will be assessed based on the inclusion criteria described below, both in the abstract reading and the full text reading steps. The eligibility criteria are broken down into the PICOC question key elements, as prescribed by the ROSES guidelines (Haddaway et al., 2017), and were developed during the pilot search phase. We will examine both primary studies and reviews. When considering reviews, we will search for the primary articles cited in the study. We will add it to the list of papers to be analysed in the cases when we can access it, otherwise we will extract the corresponding data directly from the review.

1. Population: *Articles deal with climate change mitigation*, which consists of either decreasing the amount of emissions released into the atmosphere, including carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), or reducing the current concentration of CO₂ in the atmosphere by enhancing carbon sinks (e.g. increasing the area of forests). We will only consider emissions estimates which are not outdated, setting 2010 as a threshold.
2. Interventions: *Articles explicitly mention and clearly describe a NbCS applicable in the AFOLU sector*. We will consider all mitigation strategies applicable in the AFOLU sector, including both supply-side and demand-side practices. We identified a list of interventions by Angileri et al. (2024) and further developed it during the piloting phase to adapt it to our context, described in the Supplementary materials, which we will take into consideration. This list will be further integrated if relevant practices are found during the screening stage.
3. Comparators: *Articles contain numerical estimates of the mitigation potential of one or more climate mitigation strategies or practices*. The focus is not in articles simply reporting climate emissions estimates. Thus, we will only include articles which provide the estimates of avoided emissions, or of emissions compared to a baseline scenario, and explicitly link the estimates to the adoption of a specific NbCS.
4. Outcome: *Articles model the mitigation potential of a NbCS in a specific geographical area over a certain period of time*. The focus is not on articles reporting unitarian mitigation potentials. Thus, we will only include articles providing a mitigation potential over a unit of area (e.g., hectare, km², etc.) or other uptake unit (e.g., livestock unit, etc) per year. Indeed, papers estimating a mitigation potential per unit of area usually constitute the basis for constructing the estimates on a territorial level, but this estimate is not necessarily present in the papers, therefore those papers will be excluded.

5. Context: *Articles are set in Europe*. The focus is on articles presenting climate mitigation potential models for Europe or for specific regions of the European continent, including the Mediterranean area. Therefore, we will exclude papers dealing with other regions of the world.

We will specify the number of papers excluded for each specific reason, and will provide a list of articles excluded at full text with reasons for exclusion.

3.7 Critical appraisal

In order to assess the internal validity of the studies under examination we will adopt a slightly simplified version of the grading evidence system proposed by Koutsos et al. (2019) for systematic reviews in agricultural sciences, shown in Table 3.3. Each study will be classified as having a strong, moderate or low evidence, based on the study design. Experiments, field trials and systematic reviews are considered to have substantiated evidence, whereas case studies, narrative reviews and simulations are partially substantiated. Qualitative and descriptive studies are classified as unsubstantiated and their evidence is low. Therefore, the studies presenting low evidence will be excluded from our final list of articles, and only those with strong or moderate evidence will be included in our narrative and quantitative synthesis. In order to test the repeatability of critical appraisal, around 10% of the studies will be evaluated by two or more reviewers, and every disagreement will be discussed among the team members and solved by a third party (Koutsos et al., 2019).

Following the example of Sánchez et al. (2021), we will not evaluate external validity, i.e. the generalizability of the results to other contexts, because the effectiveness of a NbCS in terms of mitigation potential is agreed by the literature to be context-dependent and can vary greatly depending on the climate, geo-morphological, chemical, physical and biological conditions of the area where it is implemented.

Table 3.3: Grading evidence system

Strength of evidence	Type of agricultural research	Study design	Study type	Evidence
Strong (I)	Applied research, Adaptive/farm level research, Strategic research, Systematic reviews and meta-analysis	Experiments, Field trials, Systematic reviews	Experimental/ Structured review	Substantiated: documented evidence

Moderate (II)	Case studies/Reviews Modeling/Simulation	Case studies, Narrative reviews, Simulations	Observational	Partial substantiated: evidence under conditions
Low (III)	Opinion papers, conference papers, workshops	Qualitative research, opinion papers, reports of expert committees	Descriptive	Unsubstantiated: potential evidence based on qualitative analysis or opinion

3.8 Data extraction

A standardised protocol was adopted for data extraction, using a structured database. The database was constructed according to the PICOC scheme so that for each paper the Population, Intervention, Comparator, Outcome and context were listed. A distinction was done between single and aggregate intervention strategies, because we expect the analysed papers to provide both the mitigation potential for single interventions, such as "no-tillage", "crop rotation", and "biochar application to soils", and for the aggregate effect of some interventions, such as "reduced tillage + crop rotation", which does not always coincide with the sum of the single mitigation potentials. In addition to these elements, the protocol prescribes to collect the bibliographical data of each paper, namely the DOI, title, abstract, year of publication, authors and, in addition to that, to provide specifications concerning the methodology employed to estimate the mitigation potentials. We will also create a unique study ID identifying each article and an intervention ID corresponding to each intervention. This choice is made with the aim of simplifying comparison between interventions in the cases in which articles compute the mitigation potentials of more than only one NbCS. The NbCS nomenclature, shown in the Supplementary materials, was homogenised across pre-screened articles during the piloting phase and official sources including Mouratiadou et al. (2024) and the recent classification of environment- and climate-related farming practices reported by the Joint Research Centre (JRC) of the European Commission (Angileri et al., 2024) in the context of the Common Agricultural Policy. Our intention is to classify the NbCS found in the literature following Angileri et al. (2024) and complementing their list of farming practices with demand-side measures. Any further development of the list will be discussed and agreed by the team, only in the case we find an intervention which does not fit into the previous classification. For each intervention in our dataset, we will code its Section, its Tier 1, Tier 2 and Tier 3 (if present) codes and names, as shown in the Supplementary materials.

The codebook presented in Table 3.4 was piloted and refined on a sample of 50 articles from the search, and the repeatability of the data extraction will be further tested by having two or more reviewers extract the data from 10% of the articles in the dataset. Any disagreement will be discussed by the team and solved by a third party. The bibliographic information will be retrieved directly from the databases Scopus and Web of Science, whereas the other variables will be either extracted as meta-data from the articles, or coded from the article texts. The variables to be coded will be Intervention category, Intervention width, Outcome category, Outcome width, Context category. All the other variables will be directly extracted from the articles, including those recording the text about the PICOC elements and the methodology (Methods text, Population text, Intervention text, Outcome text, Context text).

Table 3.4: Standardised codebook with all the variables to be extracted.

Section	Variable	Code	Dictionary options	Description
Bibliographic	Doi	doi		Article doi
	Publication year	pub_yr		Publication year of the article
	Authors	authors		Full names of the authors
	Title	title		Title of the article
	Journal reference	journal_ref		Title of the peer-reviewed journal in which the article was published
	Abstract	abstract		Full abstract
	Type of publication	pub_type	Primary article; Review.	Type of publication
	Study ID	st_id		Unique study ID identifying each article taken into consideration
	Intervention ID	int_id		Unique intervention ID identifying each intervention taken into consideration
Methodology	Methods text	met_text		Methodology adopted to estimate the mitigation potential
	Type of estimative model	met_model		Model (if present) used to estimate the mitigation potential
Population	Population text	pop_text		Description of the target of application of the mitigation intervention

	Land fraction	pop_landfr			
	Production segment	pop_prodsegm			
	Product category	pop_prodcatt			
Intervention	Intervention text	int_text			Description of the mitigation intervention for which the article assesses the mitigation potential
	Intervention section	int_section	(See materials)	Supplementary	Name of the intervention section
	Intervention Tier 1	int_t1	(See materials)	Supplementary	Name of the Tier 1 intervention
	Intervention Tier 2	int_t2	(See materials)	Supplementary	Name of the Tier 2 intervention
	Intervention Tier 3	int_t3	(See materials)	Supplementary	Name of the Tier 3 intervention
	Uptake variable	int_uptvarun	Utilised agricultural area; Livestock heads; Price of CO2 credits; Marginal land; Land; No-tillage agricultural area; Biomass (waste); Arable land; Abandoned agricultural land; Degraded and desertified soils; Irrigated soils; Rangeland and grasslands; World soils; Relative-tillage agricultural area.		Description of the land area on which the mitigation intervention is applied (agricultural land, marginal land, arable land, etc.). This list will be possibly further developed, in the case additional uptake variables are found in the articles.
	Intervention uptake unit	int_uptun	ha; Mha; km2; Gt; Mkm2; Gt/y; Gha.		Variable indicating the spatial unit of application of the mitigation intervention (Mha, ha, km2). This list will be possibly further developed, in the case additional uptake units are found in the articles.
	Uptake value	int_uptval			Number of spatial units of application
	Uptake value (%)	int_uptvalper			Fraction of uptake considered by the study over the total potential uptake (e.g. the utilisable agricultural area under the intervention over the total)
	Unitarian mitigation unit	int_unitmitun	Mg C/ha; Mg CO ₂ eq/ha; Tg C/ha/yr; Mg C /ton-biomass; Tg C/ha; g C/m;		Variable indicating the unitarian mitigation measurement unit. This list will be possibly further

			Pg C/ha/yr; Gt CO ₂ eq/yr; Mg C/ha/yr; g C/m/yr; Mg C/km ² /yr; kg Ceq/ha/yr; kg C/ha/yr; t C/ha/yr; Mt CO ₂ eq/yr; Pg C; Pg C/yr; Kg CO ₂ eq/ha; T CO ₂ eq/ha.	developed, in the case additional unitarian mitigation units are found in the articles.
	Unitarian mitigation value (mean or range)	int_unitmitval		Variable indicating the mitigation value (or range) of the intervention per spatial unit
	Year scenario	int_yr		The year/period over which the mitigation potential of the intervention is projected
Comparator	Comparator	com		Control scenario against which the effect size of the mitigation intervention is evaluated
	Reference year	com_yr		The year/period over which the mitigation potential of the control scenario is projected
Outcome	Outcome text	outc_text		Part of the article text (using authors' wording) reporting the value of the mitigation potential of the intervention
	Outcome category	outc_cat	Avoided all emissions; Avoided CO ₂ emissions; Avoided non-CO ₂ emissions; Avoided SOC release; C-sequestration; SOC sequestration; soil carbon sequestration (SCS); SOC + N ₂ O + CH ₄ + CO ₂ eq; avoided CH ₄ emission; avoided N ₂ O emission; Decrease total net GHG emissions; Increase net C sink.	Variable in which the mitigation potential is expressed. This list will be possibly further developed, in the case additional outcome categories are found in the articles.
	Outcome width	outc_width	Single; Aggregate.	Denotes whether the outcome category represent a single metric (e.g. soil carbon) or if it is the result of aggregated metrics (e.g. soil carbon + N ₂ O emission)
	Outcome unit	outc_unit		Unit amount of the outcome variable, as reported originally by the authors of each study
	Outcome mean	outc_mean		Mean value, reported in the unit amount as reported originally by the authors of each study
	Outcome CI low	outc_cilow		Lower band 95% confidence interval

	Outcome CI high	outc_cihigh		Upper band 95% confidence interval
	Outcome min	outc_min		Minimum value adopted by the outcome variable
	Outcome max	outc_max		Maximum value adopted by the outcome variable
	Outcome standard deviation	outc_sd		Standard deviation of the outcome variable
	Outcome standard error	outc_se		Standard error of the outcome variable
	Number of scenarios	outc_numb		Number of scenarios run to estimate the mitigation potential and (possibly) its range of uncertainty
	Cost value	cost_value		Value of implementation cost
	Cost unit	cost_unit	Euros	Currency used to express the implementation cost. We expect euros to be employed the most, but this list could be further developed, in the case additional implementation cost units are found in the articles.
Context	Context text	cont_text		Part of the article text reporting the regional extension of the study
	Context category	cont_cat	Continental; Regional; National; Subnational.	Scale of the study
	Context subcategory	cont_subcat		Information present when the study is concentrated in areas smaller than the national level (regions, provinces, countries, large agricultural areas etc.)

3.9 Data management

We will extract data from the articles and gather them on an excel spreadsheet. Each observation, and therefore each row on the dataset, will correspond to a single intervention. Consequently, data gathered from one study will span over multiple rows, depending on the number of alternative mitigation strategies analysed.

After the data extraction phase, we expect a large variety of interventions to be found in the reviewed articles. This variability is partly due to the use of synonyms, and partly to the consideration of interventions which are similar variations of the same strategy. Therefore, we will operate an aggregation and re-coding of the interventions, so as to have intervention macro-categories easy to compare.

To standardise the outcome measurement units, we will convert the different mass units of CO₂ in Gt, multiplying them by the required 10ⁿ factor. When needed, carbon (C) will be transformed into CO₂ equivalent by multiplying the new value expressed in Gt by 3.66. Finally, for units indicating quantities of GHGs different from CO₂, the value obtained after transforming the mass unit into Gt was multiplied by the 100-year Global Warming Potentials (GWP) of each gas (273 for N₂O and 27.9 for CH₄) to obtain the corresponding value expressed in Gt CO₂ eq/year. Similarly, in case we find implementation costs expressed in currencies other than euros, we will harmonise them to euros (Table 3.5).

Table 3.5: Standardised codebook with the variable obtained after the conversion

Variable				Code	Description
Harmonized outcome mean	(expressed in Gt CO ₂ eq/year)			outc_mean_h	Homogenised value of unitarian mitigation potential of the intervention, expressed in the unit Gt CO ₂ /ha/year (if present)
Harmonized implementation value	implementation cost	value (expressed in euros)		cost_value_h	Homogenised value of implementation cost, expressed in euros, if present

3.10 Data synthesis and presentation

A narrative synthesis will be performed in order to describe the results. Given that the articles provide adequate data, we will carry out a meta-analysis to quantify the effect of different NbCS on mitigation potential. Tables and figures will be designed to present the descriptive statistics about the distribution of the data across geographical areas, mitigation categories and mitigation strategies and quality of observation.

Results of the data analysis will display the distribution of mitigation potential estimates for each intervention category, and evaluate whether the results are consistent throughout the relevant literature while at the same time considering which interventions are estimated to have a higher and lower efficacy in mitigating climate change. We will show these results graphically by using:

a) A boxplot, which describes the distribution of the data and allows for the identification of potential outliers. For each 'box', the upper and lower bound represents the 75th and 25th percentile of the data

distribution, respectively, while the central line represents the median value of the distribution. The upper (lower) whisker, i.e., the line departing from the box, extends upwards (downwards) to the highest (lowest) value that is less than or equal to 1.5 times the interquartile range (IQR). The two whiskers show the dispersion of values below the first quartile and above the third quartile and allow the identification of potential outliers.

b) A strip plot, i.e., a one-dimensional scatterplot that allows us to look at the data distribution and, especially, to visualise the amount of data available for each category. The EU data will be discussed in more detail in relation to the most recent policies adopted by the EU on climate change mitigation.

All charts will be produced with Python (3.7 Version) using the packages *matplotlib*, *matplotlib.pyplot* and *seaborn*. We will also insert a table showing the average mitigation potential in Gt CO₂ equivalent per year computed for each NbCS, adding the standard error of the mean, and the number of estimates on which the average is computed. By looking at the number of articles analysing each NbCS, we will identify key knowledge gaps, so as to inform future research with helpful information. By writing and publishing a systematic review protocol, we reduce the publication bias of our final publication, since we set out our methodology and codebook in advance.

3.11 Bibliographic data

Figure 3.1 displays the publication year of the articles retrieved from the main search run on Web of Science and Scopus, before the screening phase. It is clear to see that the topic under examination has been the subject of a consistently increasing interest by the academic community, as depicted by the upward-sloped tendency line. The topic of mitigation potential in the European AFOLU sector was first addressed by few researchers in the 1990s. Subsequently, the volume of published articles gradually increased until the late 2010s, at which point a significant proliferation in research activity was observed, especially in the early 2020s.

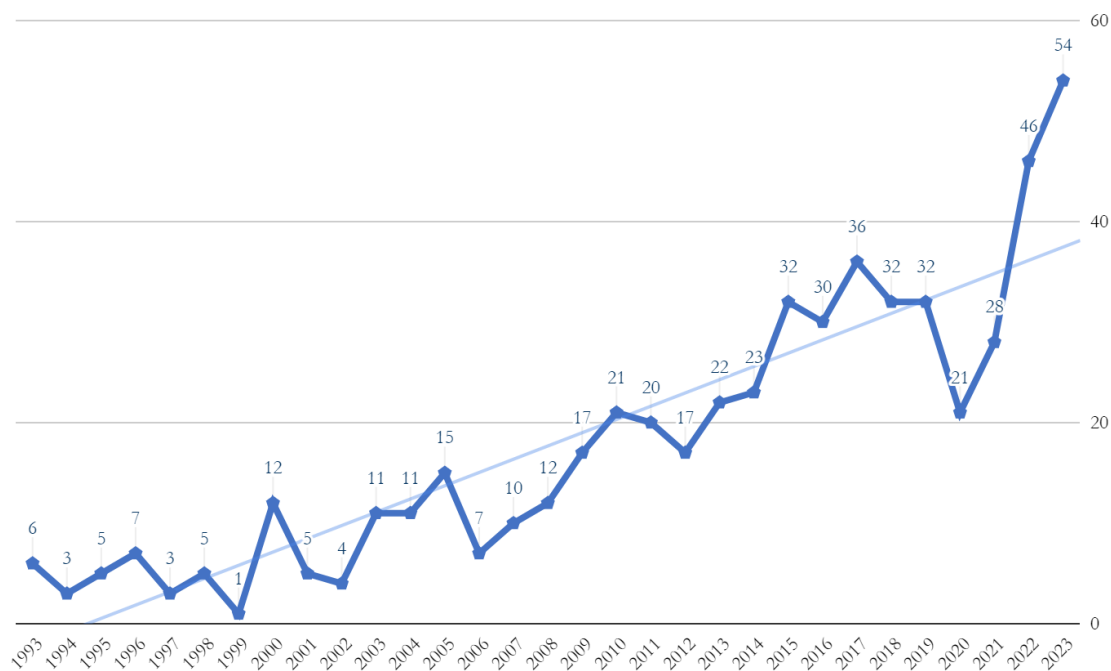


Figure 3.1: Number of articles per publication year with tendency line (548 articles retrieved from the main search). The eleven articles published in 2024 were excluded from the plotting of this graph to avoid underrepresentation.

To have an idea about which are the topics and mitigation practices most covered by these 548 articles, we selected a list of keywords and counted the number of articles mentioning them. The results are shown in Figure 3.2. The word “organic” is the most recurring one, with over 140 articles containing it. The second most recurring keyword is “grassland” (53), demonstrating a prevalence of the LULUC sector, also attested by “conservation” (37), “afforestation” (36), “conversion” (33). The keywords “livestock” (49) and “manure” (45) indicate that a high number of articles deal with animal agriculture, a very high-impact sector in terms of GHG emissions. Among the articles, 21 deal with a change in the tilling practice, 16 with agroforestry, 11 with biochar and 9 with a diet shift.

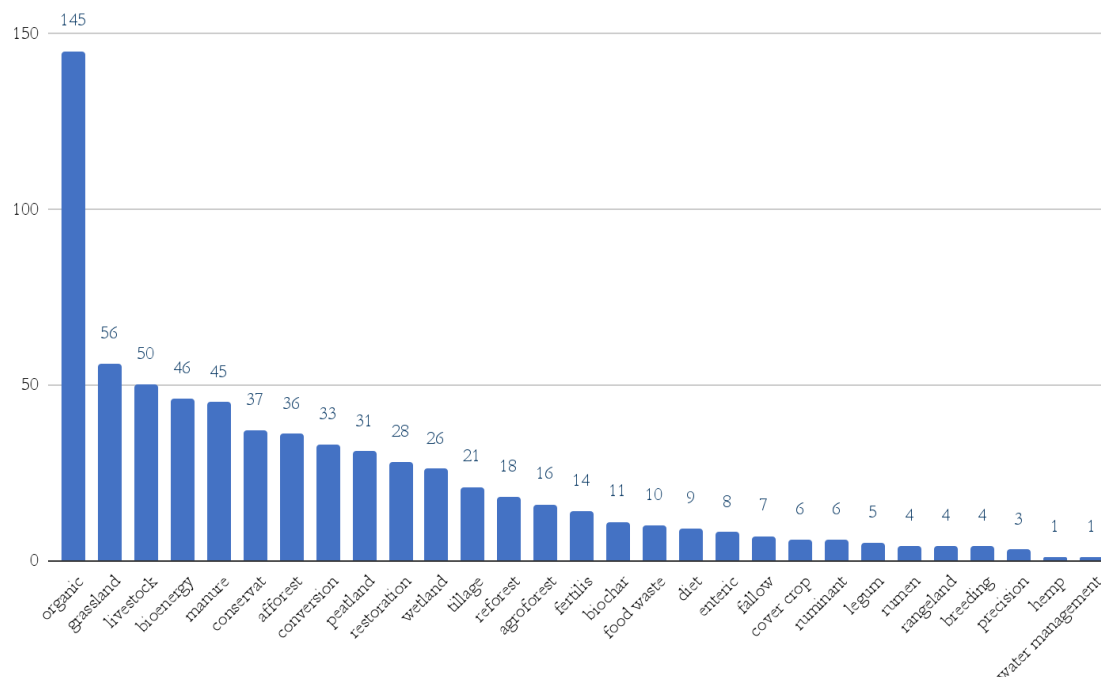


Figure 3.2: Keywords recurrence in the 573 articles retrieved through the main search.

3.12 Results overview

In the following section we present the preliminary results of a data extraction carried out on a subsample of eight articles among those selected through the main search on Web of Science. These articles include 62 interventions in total, and the results from the pilot are useful to guide the discussion.

Figure 3.3 shows the mitigation potentials data for categories available at the European level. The number of the different mitigation strategies included in the chart is 21. All values are above 0.001 Gt CO₂eq/year, with the exception of 4 data points that are below 0.001 and 3 data points that are equal to 0. Regarding data availability, there are important differences in the amount of data available for each NbCS, but they are less pronounced for the EU than in the previous charts. In fact, there are many strategies with very little data available (11 categories with 2 data and 5 categories with 3 data), while the remaining strategies report between 4 and 6 data. Regarding the data dispersion, there is great heterogeneity, with some strategies showing a more evident dispersion and others being more concentrated. There are 3 outliers, an upper and a lower one for the category "Improved fertilisation", and an upper one for "Wetlands/peatlands/coastal conservation/restoration".

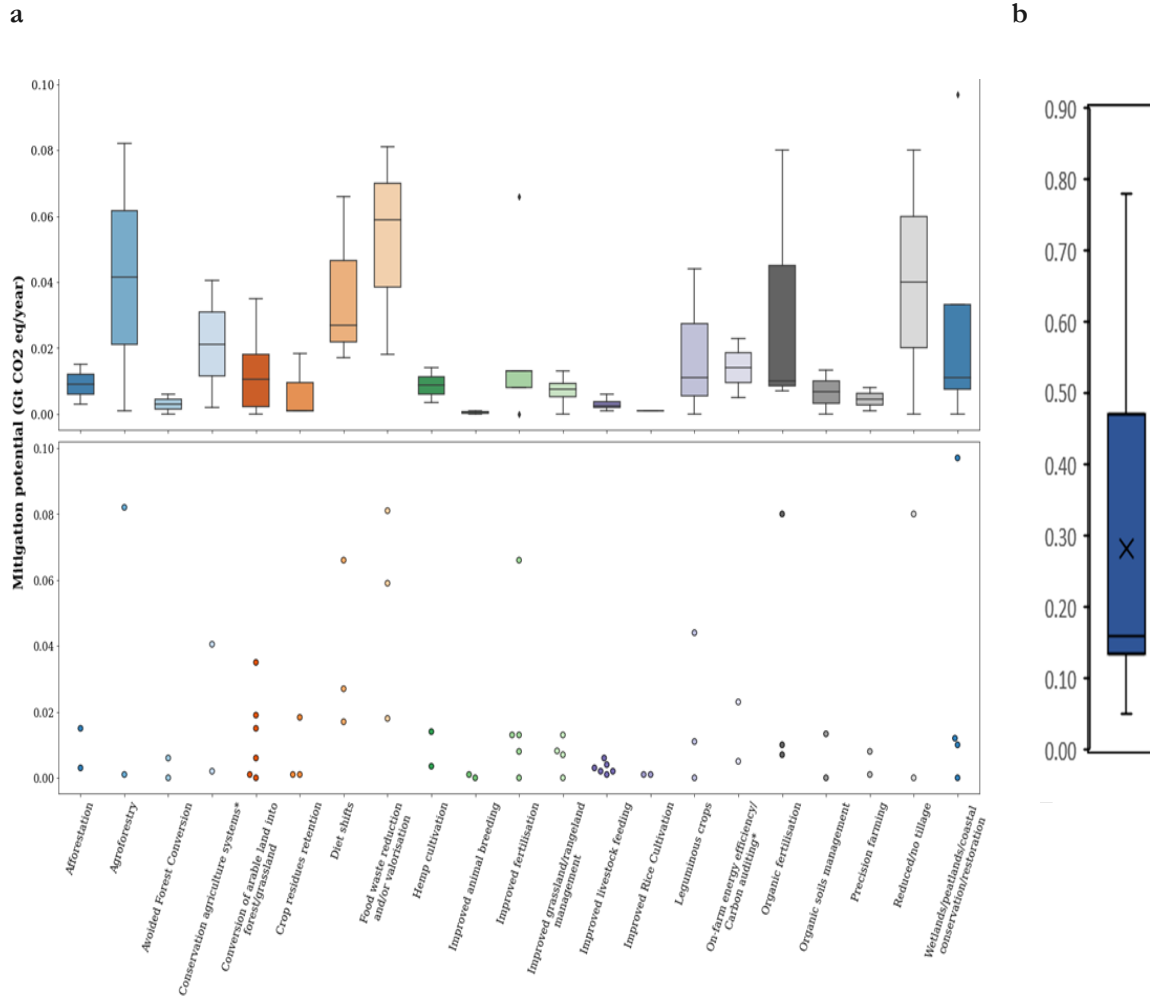


Figure 3.3. Dataplots present the pre-extracted data from a sub-sample of 14 articles. a) Boxplot reporting the mitigation potential of different NbCS at the European Union level and scatterplot showing each single point corresponding to a mitigation value. Aggregate categories are indicated with an asterisk. b) Aggregated mitigation potential estimated as cumulative effect of several NbCS applied across the EU.

Table 3.6 shows the average mitigation values calculated for each NbCS. The average mitigation impact between strategies ranges between 0 and 0.01. The NbCS with the highest mitigation potential is “Food waste reduction/valorisation”, with a mitigation value of 0.053 Gt CO₂eq/year. The strategies “Agroforestry” and “Reduced/no tillage” follow with potentials of 0.042 and 0.04 Gt CO₂eq/year, respectively. These results must be carefully considered because, for each NbCS, there are very few data points available (3, 2 and 2, respectively). The next mitigation practices in terms of high mitigation potential are “Diet shifts”, “Organic fertilisation”, and “Wetland/peatland/coastal conservation/restoration”, with values ranging between 0.03 and 0.04 Gt CO₂eq/year. It is worth noting that, according to these preliminary results, two of the most effective strategies in mitigating climate change are demand-side, which proves the relevance of including them in such analysis.

Table 3.6. Preliminary estimates of average mitigation potential (with standard error and number of data points), calculated for each NbCS retrieved during the pre-screening pilot (including 14 publications).

NbCS	Mitigation Potential (Gt CO ₂ eq/year)	Standard Error	Number of data points
Afforestation	0.009	0.004	2
Agroforestry	0.042	0.029	2
Avoided Forest Conversion	0.003	0.002	2
Conservation agriculture systems	0.021	0.014	2
Conversion of arable land into forest/grassland	0.013	0.005	6
Crop residues retention	0.007	0.005	3
Diet shifts	0.037	0.012	3
Food waste reduction/valorisation	0.053	0.015	3
Hemp cultivation	0.009	0.004	2
Improved animal breeding	0.001	0.000	2
Improved fertilisation	0.020	0.011	5
Improved grassland/rangeland management	0.007	0.002	4
Improved livestock feeding	0.003	0.001	6
Improved Rice Cultivation	0.001	0.000	2
Leguminous crops	0.018	0.011	3
On-farm energy efficiency/Carbon auditing	0.014	0.006	2
Organic fertilisation	0.032	0.019	3
Organic soils management	0.007	0.005	2
Precision farming	0.005	0.002	2
Reduced/no tillage	0.040	0.028	2
Wetlands/peatlands/coastal conservation/restoration	0.030	0.020	4
Total mitigation potential (all NbCS)	0.372	0.195	62

3.13 Discussion

The issue of climate mitigation is at the heart of the Green New Deal by the European Commission, which revises upwards the targets previously set by EU legislation and aims to make the European economy and society climate neutral by 2050. The package proposes that, by 2030, the AFOLU sector should be able to ensure an annual net GHGs uptake of 0.31 Gt CO₂eq, with specific national targets to contribute to this common target. A large part of the European climate strategy will have to be implemented via the Common Agricultural Policy (CAP), which devotes considerable resources to

encouraging farmers to adopt mitigation practices. Specifically, 40% of the CAP budget is to be dedicated to funding climate-relevant practices. Within this slice of the budget, a prominent role is played by eco-schemes, i.e., direct payments for farmers who decide to adopt environmental and climate-friendly practices. Direct payments make up 75% of the CAP budget, and at least 25% of the direct payments budget will have to be allocated to eco-schemes. The results of the systematic literature review will provide useful insights concerning the NbCS which are more promising in terms of GHG emissions reduction, and therefore which could help reach the climate mitigation targets more effectively, if incentivised by the European commission and the national governments.

Our preliminary results show that agroforestry and reduced/no tillage are amongst the NbCS with higher mitigation potentials. These practices are already incentivised by the legislation, and could further be supported in future reforms of the CAP. A contradiction lying in the design of the CAP is also brought to light: organic farming, which is the primary target of CAP eco-schemes, results to have one of the lowest mitigation potentials according to our data. This could potentially entail that funding would find more effective applications in other farming practices, provided that these results will be corroborated by the final results of the systematic review. Interestingly, data show that food waste reduction/valorisation and diet shifts are projected to be highly effective mitigation measures, suggesting that significant more attention should be paid to demand-side measures, both by academia and by policymakers. In conclusion, the final results of the systematic review will show if the NbCS have the potential to adequately contribute to EU climate goals, if properly supported by policymakers.

CHAPTER 4 BIOSTIMULANTS IN RICE, A POSSIBLE WAY FOR SUSTAINABLE FERTILIZATION

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Abstract

Introducing smart and sustainable tools for climate change adaptation and mitigation is a major need to support agriculture's productivity potential. We tested a consortium of biostimulants during two seasons (2023-2024). During these two years a biostimulant composed of the following components was evaluated in rice fields: *Azobacter chroococcum*, *Azospirillum brasiliense*, *Bacillus velezensis*, with foliar fertilizer of fermented sugarcane. The biostimulant was tested separately on two rice varieties (Carnaroli and Rosa Marchetti) with green mulching as field management. Data collection on biomass and LAI measures were taken in replicates during the rice physiological stages: tillering, elongation, flag leaf and yield at the harvest time. The use of these nitrogen-fixing biostimulants represents a significant challenge for agriculture, especially for open-field cereals, particularly when considering the European Union's goals to reduce the use of synthetic chemical nitrogen in agriculture

4.1 Introduction

Rice (*Oryza sativa* L.) provides approximately 20% of the caloric intake for over half the world's population. In the Mediterranean basin, rice cultivation spans approximately 1.3 million hectares. Italy stands as the leading rice producer in Europe, accounting for more than half of the EU's total rice production and maintaining a high standard of quality. It is also the second-largest rice producer in the Mediterranean region, following Egypt.

The primary Italian rice-growing region is the Po Valley, specifically the portion on the left bank of the Po River and along the Ticino River, which crosses the regions of Lombardy and Piedmont in northern Italy. This area comprised approximately 213,000 hectares in 2017, representing 93% of Italy's total rice production area, as reported by the Ente Nazionale Risi.

Rice production in Italy is concentrated in the northern regions, with Piedmont and Lombardy leading in production and market share. About 90% of the rice area in Italy is concentrated in the provinces of Pavia, Vercelli, and Novara. Specifically, the Italian region with the largest area devoted to rice cultivation is Piedmont (51% of the national rice area), followed by Lombardy with 42% (source: Ente Nazionale Risi). In Lombardy, rice production is concentrated in the province of Pavia, where various rice varieties, including Carnaroli, Arborio, and Vialone Nano, are produced in over fifty municipalities. The province of Pavia is the Italian province with the largest rice area, approximately 70,000 hectares, contributing to over 35% of the Italian rice area. In Lombardy, rice cultivation also involves the lower province of Milano and Mantova, where specific production districts have been established for this crop. The pursuit of high yields involves the use of technical means (including nitrogen fertilizers, e.g., urea, and herbicides, e.g., glyphosate) which, if not properly managed, can lead to increased production costs and environmental impact, especially due to the low nitrogen use efficiencies ($< 30\%$) (Baral et al., 2020) on average quantities distributed, which in Italy often exceed 150 kg N ha^{-1} , commonly observed in this particular agroecosystem and due to the peculiar presence of spontaneous herbaceous species (in Italy mainly wild rice, barnyard grass, sedges, heteranthera) due to water resource management (Dass et al., 2017). Fertilization and crop protection represent the main costs for rice farms. Specifically, in Italy, the cost of purchasing fertilizers represents over 15% of total farm costs for medium-sized farms with owned land and almost 40% of the total cost for the purchase of production factors. Regarding interventions for protection, the corresponding values are over 10% (of farm costs) and over 25% (of production factors' costs), with weed control – chemical (e.g., sulfonylureas, imidazolinones) or mechanical – playing the main role. These figures clearly demonstrate how optimizing fertilizer management and protection interventions (especially weed control treatments; Ahmed et al., 2021; Movedi et al., 2022) would improve the profitability of companies, environmental sustainability, and therefore, the competitiveness of the entire sector.

The goal of this study is to test biostimulants in organic rice cultivation, in order to verify, through various agronomic parameters (LAI, biomass, and yield), any effectiveness of the products that could lead to their integration also into conventional rice farming practices, thereby reducing the use of nitrogen units from chemical fertilization, in line with what Europe is advocating.

In Europe, the EBIC (European Biostimulants Industry Council) defined plant biostimulants as follows. “Plant biostimulants contain substance(s) and/or microorganisms whose function when applied to plants or to the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality. Biostimulants have no direct action against pests, and therefore do not fall within the regulatory framework of pesticides”.

The EBIC later elaborated on the effects that biostimulants can have on plants as follows: “Biostimulants foster plant growth and development throughout the crop life cycle from seed germination to plant maturity in a number of demonstrated ways, including but not limited to: improving the efficiency of the plant’s metabolism to induce yield increases and enhanced crop quality; increasing plant tolerance to and recovery from abiotic stresses; facilitating nutrient assimilation, translocation and use; enhancing quality attributes of produce, including sugar content, colour, fruit seeding, etc.; rendering water use more efficient; enhancing certain physicochemical properties of the soil and fostering the development of complementary soil micro-organisms” (Calvo et al., 2014).

Microbial inoculants that function as biofertilizers are recognized as biostimulants. These biofertilizers are biological products containing live microorganisms that, when applied to seeds, plant surfaces, or soil, enhance plant growth through mechanisms such as increasing nutrient availability, boosting root biomass or surface area, and improving nutrient uptake efficiency (Vessey, 2003). They can serve as complementary tools alongside mineral fertilizers (Canbolat et al., 2006).

Recent studies have highlighted the presence of nitrogen-fixing bacteria (N₂-fixers) and nitrogenase activity in various plant-associated habitats, such as the xylem sap and epiphytic phyllosphere of maize (Zhang et al., 2022; Abaldi et al., 2021), the leaf endosphere of pioneer plants like *Salix rehderiana*, *Salix ernestii*, and *Populus purdomii* (Sun et al., 2022), forest canopy leaves (Moreira et al., 2021; Stanton et al., 2019), and mosses in high-latitude ecosystems (Zhu et al., 2022). These findings suggest that the phyllosphere offers favorable conditions for diazotrophic bacteria capable of fixing atmospheric nitrogen. These bacteria utilize carbohydrates excreted by plant leaves (Vorholt, 2012) and contribute significant nitrogen, potentially benefiting plant growth (Murty, 1984; Abril et al., 2005).

The importance of exploring biological nitrogen fixation (BNF) in the phyllosphere lies in its potential to identify key N₂-fixing bacteria in terrestrial ecosystems, which could enhance our understanding and management of global nitrogen cycling. Leaves, as the primary interface between plants and the atmosphere, host abundant populations of free-living diazotrophs, making them a critical site for nitrogen input into terrestrial systems (Zhu et al., 2022). These bacteria can fix approximately 10 kg N ha⁻¹ year⁻¹ in many ecosystems, collectively accounting for a significant share of global nitrogen fixation (Cleveland et al., 2010). Phyllosphere-associated BNF typically occurs on the leaf surface (episphere) rather than within the leaf tissue (endosphere) (Furnkranz et al., 2008). For instance, substituting half of the urea application with nitrogen-fixing cyanobacteria during rice cultivation led to higher nitrogen retention in paddy soil at the end of the growing season compared to full urea fertilization (Song et al., 2021). Thus, assessing BNF rates and identifying key nitrogen-fixing bacteria in leaves are essential for sustainable nitrogen management.

Among diazotrophs, a high relative abundance of *Azospirillum* in the phyllosphere of herbaceous plants corroborates findings that grasses and forbs often host this genus in both leaves and roots (Hurek et al., 2003; Steenhoudt et al., 2000). However, it remains unclear whether diazotrophic diversity and abundance vary between crop varieties. *Azospirillum* spp. have been widely studied for their capacity to fix atmospheric nitrogen. Using the ^{15}N tracer method, *Azospirillum brasilense* and *Azospirillum lipoferum* were shown to contribute 7–12% of wheat's total nitrogen content (Malik et al., 2002), while nitrogen fixation by *Azospirillum diazotrophicus* provided 60–80% of sugarcane's nitrogen (Boddey et al., 1991). Significant nitrogen increases due to *Azospirillum* inoculation have also been reported in cotton, wheat, sugarcane, and maize (Garcia de Salamone et al., 1996; Fayez and Daw, 1987; Saubidet et al., 2000).

The ancient coevolution between plants and their phytomicrobiome reflects a dynamic relationship shaped over millions of years. This evolutionary interplay has fostered beneficial plant-microbe interactions that can greatly impact global food production. While the phytomicrobiome holds immense promise for sustainable crop management, our understanding of its potential remains limited, and further exploration is needed (Backer et al., 2021).

The nitrogenase enzyme, responsible for nitrogen fixation, typically consists of molybdenum–iron cofactors. Some bacteria, such as *Azotobacter* and *Rhodobacter*, can synthesize alternative nitrogenases containing vanadium–iron or iron–iron cofactors under conditions of molybdenum scarcity (Dixon and Khan, 2004). However, nitrogenase is highly sensitive to oxygen, which poses a major constraint for phyllosphere-associated diazotrophs (Bennet et al., 2020). These microorganisms have developed various strategies to mitigate oxygen toxicity, including anaerobic metabolism, rapid oxygen consumption, creating diffusion barriers, or spatial and temporal compartmentalization of nitrogenase (Dixon and Khan, 2004; Bennet et al., 2020). For instance, *Azotobacter vinelandii* and *Klebsiella pneumoniae* use uncoupled cytochrome bd oxidase to protect nitrogenase, while *Nostoc* spp. form specialized heterocysts to create microaerobic conditions (Bennet et al., 2020).

Environmental factors such as light, temperature, nitrogen deposition, and precipitation significantly influence phyllosphere-associated N_2 -fixers (Furnkranz et al., 2008; Taylor et al., 2018). For example, temperature and molybdenum availability positively influence BNF in mosses (Renaudin et al., 2022), whereas warming reduces moss cover and decreases BNF activity (Permin et al., 2022). Human activities, such as urbanization, can alter the biodiversity and function of phyllosphere diazotrophs (Li et al., 2019). Moreover, microbial interactions are critical for N_2 -fixers' activity. Synergistic interactions, such as between *Bradyrhizobium* spp. and *Azospirillum baldaniorum*, have been shown to mitigate stress and enhance nitrogen fixation (Lopes et al., 2022).

Innovative strategies, including microbial inoculants and synthetic microbiomes, could improve nitrogen use efficiency in agriculture (Albright et al., 2022). However, achieving successful colonization remains a

significant challenge due to the host selectivity of N₂-fixers. Advanced tools like PANOMICS (Weckwerth et al., 2020), single-cell omics, Raman spectroscopy with stable isotope probing (Cui et al., 2018), and CRISPR-based genomic editing (Koch, 2022) may offer new insights into plant-microbe interactions.

To meet the increasing demand for food, leveraging plant-growth-promoting microorganisms (PGPMs) as biofertilizers is crucial (Parasuraman et al., 2019). Among PGPMs, nitrogen-fixing bacteria hold particular promise for improving plant growth and soil nitrogen input (Albino et al., 2006). Foliar application of nitrogen-fixing bacteria represents a viable alternative to chemical fertilizers and could play a pivotal role in promoting sustainable agriculture.

4.2 Materials and Method

4.2.1 Experimental site and treatments

The field trial was located in Rovasenda (45°32'N 8°19'E) and was performed in a silty-loam soil. The local climate is temperate (Cfb as Köppen classification), with an average annual temperature of 13°C and annual precipitation of 1290 mm. The soil (sand 24%, clay 13%, silt 63%, SOM content 2,34% in the layer 0-40 cm) is zero calcareous and acidic (pH 5,36), and it has a cation exchange capacity equal to 3,30 meq 100 g⁻¹. During the experiment, climatic data were collected from an automated meteorological station positioned close to the field (Fig. 4.1).

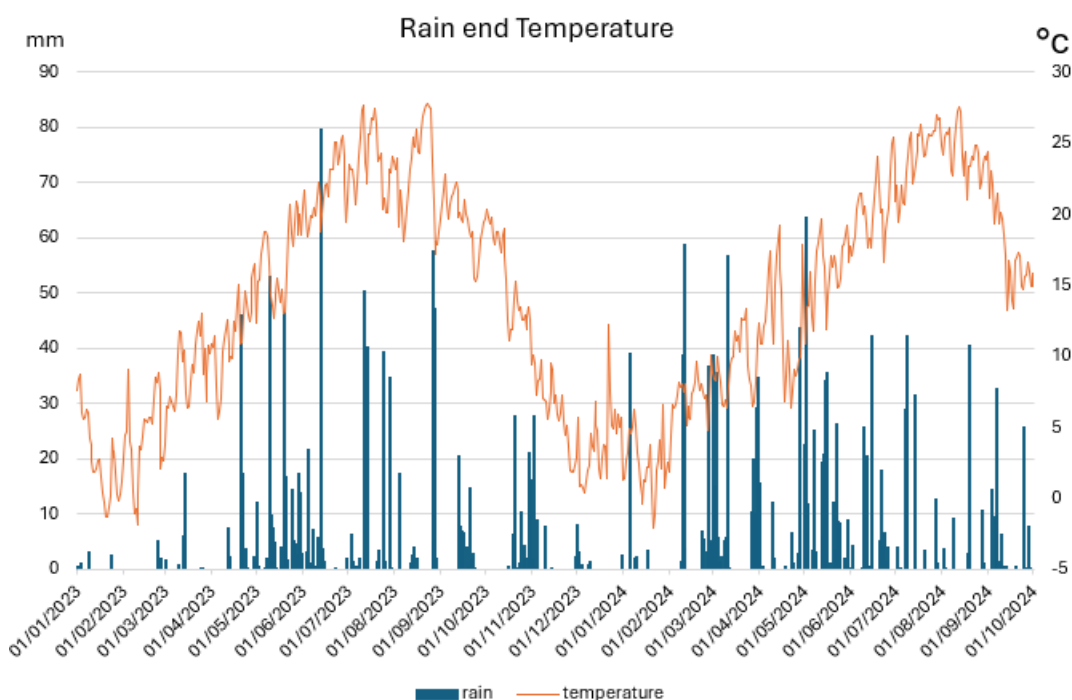


Figure 4.1: meteorological trend of temperature and rainfall during 2023 and 2024

Over the two years of field experimentation (2023 and 2024), a biostimulant composed of three microorganisms was tested: *Azotobacter chroococcum*, *Azospirillum brasiliense*, and *Bacillus velezensis* ($5,5 \times 10^9$ UFC/g). Additionally, a foliar fertilizer based on fermented sugar cane (NK 1,4-4,7) was added as a substrate for the microorganisms. The treatment mixture is composed of 0,5kg of biostimulant and 4 liters of foliar fertilizer, and was distributed with the spray barrel.

The treatment was tested separately on two rice varieties (Carnaroli and Rosa Marchetti). A randomized block design was arranged for each rice variety to test crop responses to the biostimulant treatment. The treatment factor included two levels, treated and untreated (consisting in a control treatment with no biostimulant application), that were repeated in each rice variety field. Each plot of the randomized block designs had a 1-hectare area (Fig. 4.2). The treatment was carried out, during each season, at the phenological stage of the first step of stem elongation just after full tillering, when the plant reaches the maximum number of tillers (BBCH 30).



Figure 4.2: Plot arrangement

The previous crop was soybean. The agronomic method adopted by the farm is based on the green mulching, which involves planting a cover crop before rice sowing, suppresses weeds through competition, mulching, allelopathy, and the toxicity of fermented organic matter. *Lolium multiflorum* (50 kg/ha) was sown in autumn. At the end of April, water was introduced, and rice was broadcast-seeded directly onto the cover crop to exploit the allelopathic effect against weeds. in the Table 4.1 describe in detail the adopted practice.

OPERATION	INPUT	PRODUCT	AMOUNT	UNIT	FUEL	UNIT
Plowing			1		70	l/ha
Fertilization	Hoof and horn	Nitrogen 14%	250	kg/ha	10	l/ha
Harrowing			2		25	l/ha
Levelling			1		12	l/ha
Sowing	Seed	<i>Lolium multiflorum</i> seed	50	kg/ha	10	l/ha
Fertilization	Potassium sulfate	Potassium 50%	180	kg/ha	10	l/ha
Sowing	Seed	Carnaroli rice seed	180	kg/ha	10	l/ha
Fungicide	Thiopron	Sulfur	5	l/ha	36	l/ha
Fungicide	Thiopron	Sulfur	5	l/ha	36	l/ha
Fungicide	Thiopron	Sulfur	5	l/ha	36	l/ha
Harvest			2	ton/ha	50	l/ha

Table 4.1: agronomic practices conducted in the fields

4.2.2 Direct measures

For each plot, five randomized sampling points were chosen to perform direct measures. LAI were collected using the Pocket LAI application (Confalonieri et al., 2013), by performing five measures replicated for each sampling point.

At each sampling point, crop aboveground biomass was measured by harvesting 1 square meter of plants, and grain yields were recorded at the harvest time. The three field surveys were carried out at the following phenological stages:

- Maximum number of tillers (BBCH 29) (before treatments)
- Internode elongation (BBCH 34)

- Late boot stage (BBCH 45)

To measure the dry matter content (DM, %), samples of yield and residues were oven-dried at 105 °C until constant weight. In case of fresh samples weighing more than 1 kg m⁻², only a representative sub-sample was withheld to determine dry weight.

4.2.3 Statistical analysis

LAI, biomass, and yield data outliers were removed, before the statistical analysis, by using Grubbs test (Grubbs, 1950) from the R package “outliers” (Komsta, 2022). The test was applied to each combination of season*field survey*block*treatment to eliminate outliers of the three dependent variables.

Analysis of variance (ANOVA) to test the effect of biostimulant treatment on the variables measured repeatedly over time (aboveground biomass and LAI) was performed separately for each variable and each growing season, by means of a linear mixed model (Laird and Ware, 1982). Therefore, the model included four factors (biostimulant treatment, growing season, field survey, block) and one interaction (biostimulant treatment * field survey). The subject on which the repeated measurements were performed was represented by the plot within each growing season. For these models, variance-covariance structures have been chosen on the base of the assessment of the fitted values versus model residuals plot.

A linear model was instead applied for the ANOVA of the dependent variables measured only once during each growing season (yield), by analyzing growing season separately. In this case the linear model included two factors (biostimulant treatment and block). All analyses were conducted with R ver. 4.0.5 (Team, 2013).

4.3 Results

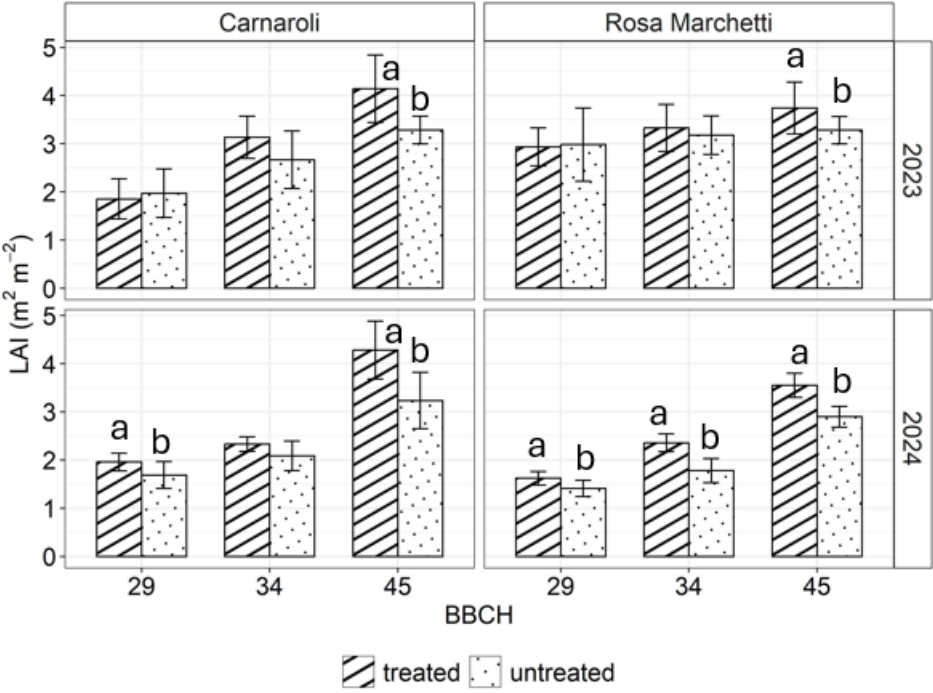


Figure 4.3: mean LAI data during three different BBCH and statistical significance

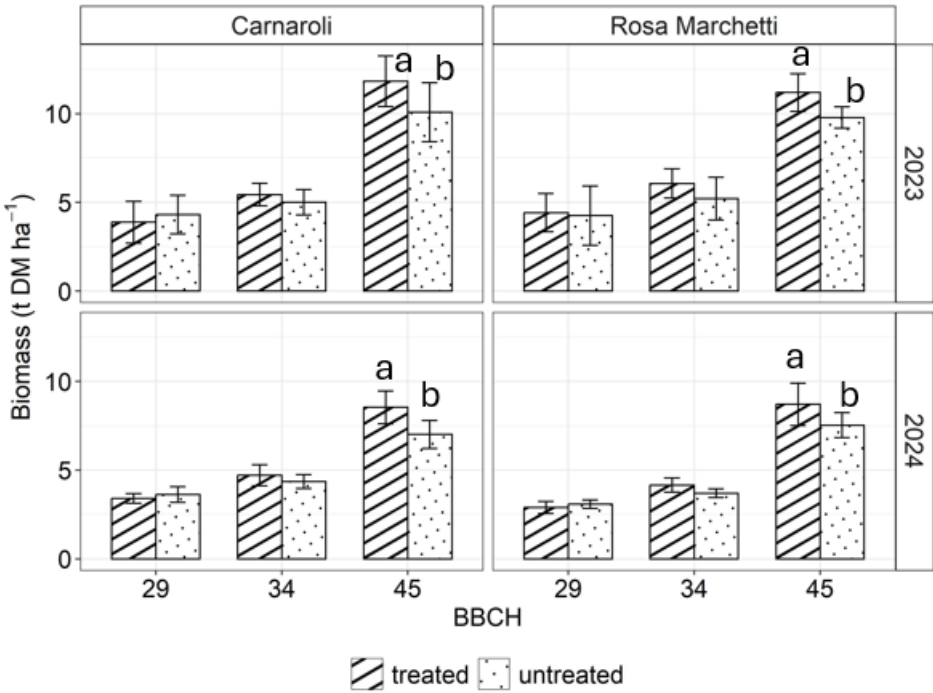


Figure 4.4: mean Biomass data during three different BBCH and statistical significance

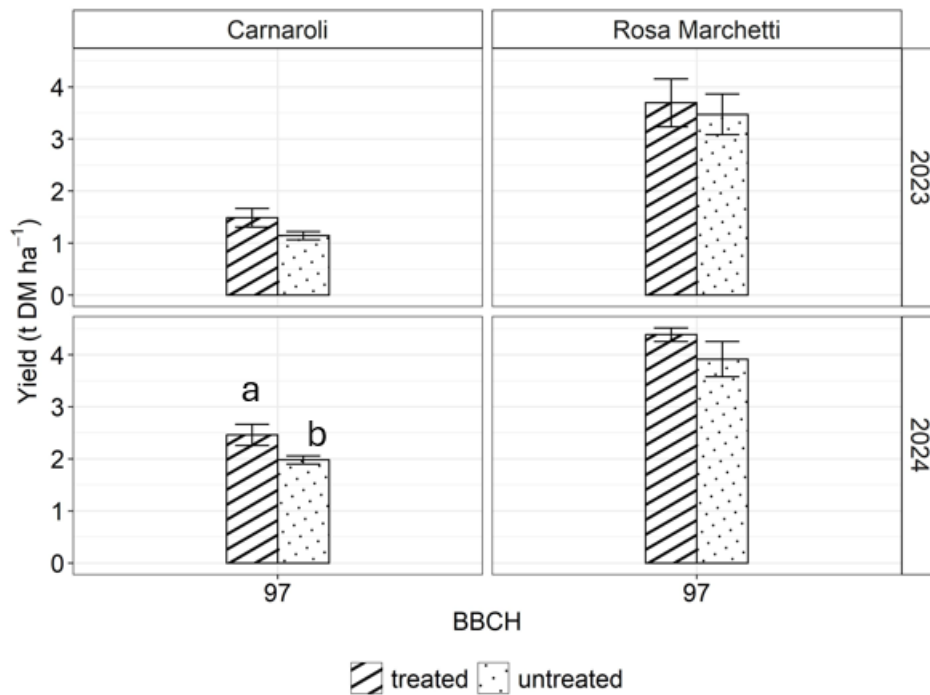


Figure 4.5: mean Yield data during two different years and statistical significance

As illustrated in the images, higher data are shown both in terms of LAI and biomass in the two varieties and over the two years for the different BBCH stages. Particularly in the survey conducted at BBCH 45, it is noted that the averages for both LAI and Biomass have significant differences depending on the Treatment. This means that the treatment works well in terms of plant growth. This point can be very interesting, because by increasing its size the rice plant can compete more with weeds and therefore be a very efficient weapon for more sustainable rice cultivation. This trend of increasing data with the treatment can also be observed with the yield, even if in this case only Carnaroli 2024 is significant, consequently it can be stated that for the yield we still do not have sufficient data to see a direct relationship with the treatment.

4.5 Conclusions

In conclusion, rice cultivation, particularly in the Mediterranean and Italian regions, remains vital for global food security and local economies. However, the significant reliance on chemical fertilizers and pesticides in conventional farming poses both environmental and financial challenges, necessitating

sustainable alternatives. This study highlights the potential of biostimulants and biofertilizers, such as nitrogen-fixing bacteria, to enhance nutrient use efficiency and reduce dependency on synthetic inputs. By promoting biological nitrogen fixation, specifically in the phyllosphere, and improving soil and plant health through the targeted use of microorganisms, these approaches could play a key role in advancing sustainable agricultural practices. Furthermore, optimizing microbial strategies and leveraging genetic engineering tools can foster more resilient agricultural ecosystems. The integration of these biotechnological innovations into rice farming not only holds promise for reducing environmental impacts but also aligns with global efforts towards sustainable food production and increased crop resilience in the face of climate change.

The results of this study are very promising but not sufficient; further studies are needed on more varieties and in different areas. Additionally, future field studies should be accompanied by laboratory studies to better understand the mechanism of action of these microorganisms.

CHAPTER 5 THE POTENTIAL SCALING UP OF ALLELOPATHIC RICE (ORYZA SPP.) USE FOR SUSTAINABLE AGRICULTURE.

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Abstract

The continuous and intensive use of phytosanitary products, both globally and regionally, poses risks to ecosystem health. This issue arises when the presence of various compounds from these products exceeds the legal limits set for surface and groundwater. Total herbicides are consistently found in water sources throughout the Lombardy Region, often in combination with other compounds. More specific products, such as Bentazone, which was historically used primarily as a selective herbicide for rice, have a more defined distribution area due to their specialized application. In recent years, numerous studies have been conducted to identify eco-friendly alternatives to rice cultivation methods that still rely heavily on phytosanitary products. One of these alternatives involves the use of allelopathic rice varieties. These varieties have a natural ability to compete with weeds by releasing biochemical compounds into the rhizosphere, which inhibit the germination or growth of these unwanted plants.

This paper presents both the results of an extensive literature review on allelopathic rice varieties known and used worldwide, and a description of the main weed species in rice fields, with particular reference to the cultivation area in Northern Italy. Based on this research, seed replication plots of allelopathic rice varieties were established to produce seeds for future field studies. The seeds for these plots were primarily obtained from the IRRI germplasm bank.

5.1 Introduction

Managing weeds in the rice paddy is essential to guarantee adequate production levels. Studies confirm that weeds compete highly with the crop for space, light, water, and nutrients. This is accentuated by the fact that wild plants can grow and develop earlier and faster than rice, compromising their final yield if not adequately controlled (Dass et al 2017). The weeds present in the paddy field are specialised for the specific cultivation environment. Weed management is complex and requires a good knowledge of plant species. Weed control is carried out with soil tillage and agrotechnical methods for the uprooting and killing weeds, and phytosanitary products that are selective on the crop are used. The use of plant protection products in rice fields is particularly studied and is important due to environmental repercussions relating to the water-soil-air compartments (Falzarano, 2020). In recent years as regards the availability of technical such the use of pesticides in rice cultivation, more than in other crops, is facing a progressive decrease of phytosanitary molecules that can be used for the control of weeds and, above all, a reduced number of available mechanisms of action. Furthermore, the prolonged and repeated use of some herbicides with the same mechanism of action has exerted a strong selection pressure on weeds by selecting populations of weeds resistant to these herbicides.

Organic rice farming is a cultivation method that involves the valorisation of the farm's internal resources without resorting to the use of synthetic plant protection products, thus favoring the varietal choice, the adoption of different crops using rotations to improve the fertility of the land, practices suitable for the containment of weeds in the rice paddy, all suitably combined about the specificity of the territory, to finally obtain optimal productions.

Since this cultivation system has a certain complexity of implementation, the diffusion of organic rice cultivation has always been limited. Organic rice cultivation has encountered a lot of skepticism in the environment of agricultural entrepreneurs as it appears very difficult to manage the weeds of rice fields without the use of chemical products, given the presence of high humidity in these areas, which allows the development of a flora capable of severely penalise the harvest. For this reason, eco-compatible techniques are implemented to contain its development within acceptable limits, which manage to avoid exceeding the harmfulness threshold, beyond which it is no longer convenient to produce for economic reasons. Practices of this kind can be, for example, false sowing, which consists of carefully preparing the soil without sowing, weeding out weeds as they germinate, and finally, sowing rice. Techniques such as dry sowing are also used, allowing mechanical weeding before submersion, green mulch with plants grown before rice and ground rolled, and the transplantation of rice seedlings in specialised nurseries.

At a global level as well as in Europe, the use of agrochemicals beyond legal limits leads to situations where soil and water monitoring show quality values considered "poor". Products like Bentazone, primarily used as a selective herbicide in rice cultivation, have a well-defined distribution area strongly

related to their main use (rice-growing areas in the province of Pavia in Italy). Over time, extensive use of phytosanitary products in rice cultivation consequently causes environmental problems. To address the issues related to the widespread use of phytosanitary products in recent years, alongside the evolution of rice cultivation and the increasing demands of the market, it is deemed necessary to find new alternative solutions to reduce dependency on these products in rice ecosystem. (Kong et al., 2011). Therefore, the trend is to seek weed control systems through scientific trials, capable of utilizing plant natural inhibitors in a completely economically sustainable manner for growers, especially in those countries where mechanical and chemical interventions are too costly. (Kato-Noguchi et al., 2011). For all these reasons, there has been a growing interest in studying allelopathic traits present in various rice varieties cultivated in rice-growing areas worldwide.

The term allelopathy, as defined by the International Allelopathy Society in 1996, following the reformulation of previous descriptions of this phenomenon, refers to any process involving secondary metabolites produced by plants, microorganisms, viruses, and fungi that influence the growth and development of agriculture and biological systems (excluding animals), encompassing both positive and negative effects (Amb et al., 2016).

Today, there is abundant information available in this area of biology, as many of these compounds found in nature have been isolated and characterized.

Allelopathic rice cultivars and species can therefore be used to harness the release of these natural "herbicides," also called allelochemicals, to inhibit the growth of weeds, providing a new option for sustainable weed management and an alternative to conventional agriculture (Gaofeng et al., 2018).

Rice, playing a significant role in agriculture, has long been studied to extract its allelopathic traits, which have been found in its extracts, root exudates, and decomposing residues (Kato-Noguchi et al., 2013); these chemical compounds are also released into the environment through volatilization and leaf leaching, making this cereal one of the most suitable crops for utilizing this phenomenon (Jensen et al., 2008).

Following research, it has been found that approximately 4% of rice varieties on Earth have exhibited allelopathic potential against some of the most harmful weeds, such as *Echinochloa crus-galli*, *Cyperus difformis*, and *Heteranthera limosa*. (Alam et al., 2015).

Furthermore, the variety and origin of rice can regulate allelopathic behavior; for example, cultivars belonging to the Japonica subspecies have shown greater weed suppression compared to Indica-type varieties and hybrids between these two types. (Khanh et al., 2007).

Many of these compounds released into the soil are secondary metabolites and include alkaloids, phenols, flavonoids, terpenes, glucosinolates that do not strongly participate in the plant's primary metabolism but are essential for its survival. Additionally, cytokinins, fatty acids, indoles, momilactones, phenolic acids, steroids, and other constituents are also included (Khanh et al., 2007).

Table 5.1: Main compounds released by allelochemical varieties (Khanh et al., 2007)

Chemical classification	Identified constituents	Sources	Selected references
Cytokinins	Cytokinins	Root exudates	Soejima <i>et al.</i> (1992)
Fatty acids	Stearic acid	Rice soil	Mattice <i>et al.</i> (1998)
Indoles	Azelaic acid	Root exudates	Rimando <i>et al.</i> (2001)
	Indoles (1H-indole-3-carboxaldehyde; 1H-indole-3-carboxylic acid; 1H-indole-5-carboxylic acid)	Root exudates	Rimando <i>et al.</i> (2001)
	Indole-5-carboxylic acid	Root exudates	Seal <i>et al.</i> (2004b)
Momilactones	Momilactone A and B	Rice hulls	Cartwright <i>et al.</i> (1981)
		Rice hulls	Chung <i>et al.</i> (2005a)
		Leaves and straw	Kato <i>et al.</i> (1973)
		Root exudates	Kato-Noguchi & Ino (2003, 2005)
Phenolic acids	Benzoic acid	Straw	Kuwatsuka & Shindo (1973)
	Caffeic acid	Straw	Kuwatsuka & Shindo (1973)
		Root exudates	Seal <i>et al.</i> (2004a)
	Ferulic acid	Decomposed straw	Chou & Lin (1976)
		Rice soil	Chou & Chiou (1979)
		Leaves and stems	Chou <i>et al.</i> (1991)
	<i>m</i> -Coumaric acid	Leaves and stems	Chou <i>et al.</i> (1991)
	<i>o</i> -Coumaric acid	Leaves and stems	Chou <i>et al.</i> (1991)
	<i>p</i> -Coumaric acid	Decomposed straw	Chou & Lin (1976)
		Leaves and stems	Chou & Chiou (1979)
		Rice soil	Rimando <i>et al.</i> (2001)
		Root exudates	Seal <i>et al.</i> (2004a)
	<i>t</i> -Coumaric acid	Root exudates	Seal <i>et al.</i> (2004a)
		Straw	Kuwatsuka & Shindo (1973)
	Gallic acid	Leaves and stems	Chou <i>et al.</i> (1991)
	Gentisic acid	Straw	Kuwatsuka & Shindo (1973)
	<i>t</i> -Ferulic acid	Root exudates	Seal <i>et al.</i> (2004a)
		Decomposed straw	Chou & Lin (1976)
		Leaves and stems	Chou <i>et al.</i> (1991)
	<i>p</i> -Hydroxybenzoic acid	Rice soil	Olofsdotter <i>et al.</i> (2002b)
		Root exudates	Seal <i>et al.</i> (2004a)
	<i>p</i> -Salicylic acid	Decomposed straw	Chou & Lin (1976)
	Protocatechuic acid	Straw	Kuwatsuka & Shindo (1973)
		Leaves and straw	Chou <i>et al.</i> (1991)
	Salicylic acid	Straw	Kuwatsuka & Shindo (1973)
		Root exudates	Seal <i>et al.</i> (2004a)
	Mandelic acid	Decomposed straw	Chou & Lin (1976)
	Sinapic acid	Straw	Kuwatsuka & Shindo (1973)
	Vanillic acid	Decomposed straw	Chou & Lin (1976)
		Rice soil	Mattice <i>et al.</i> (1998)
		Root exudates	Seal <i>et al.</i> (2004a)
	Syringic acid	Straw	Kuwatsuka & Shindo (1973)
		Rice soil	Chou & Chiou (1979)
		Decomposed straw	Chou <i>et al.</i> (1977)
		Root exudates	Seal <i>et al.</i> (2004a)
Steroids	Stigmastanols (-3 β - <i>p</i> -glyceroyldihydrocoumaroate and -3 β - <i>p</i> -butanoyldihydrocoumaroate)	Rice hulls	Chung <i>et al.</i> (2006a)
	Ergosterol peroxide and 7-oxo-stigmasterol	Fresh roots, plant parts of rice	Macias <i>et al.</i> (2006)
Other constituents	1,2-Benzenedicarboxylic acid bis (2-ethylhexyl)ester	Root exudates	Rimando <i>et al.</i> (2001)
	2-Methyl-1,4-benzenediol	Root exudates	Kim & Kim (2000)
	1-Phenyl-2-hydroxy-3,7-dimethyl- 11-aldehydic-tetradecane-2- β -D-glucopyranoside	Rice hulls	Chung <i>et al.</i> (2006a)
	3-Hydroxy-4-methoxybenzoic acid	Rice soil	Mattice <i>et al.</i> (1998)
	3-Isopropyl-5-acetoxycyclohexene-2-one-1	Root exudates	Kong <i>et al.</i> (2004a)
	4-Ethylbenzaldehyde	Root exudates	Mattice <i>et al.</i> (1998)
	2- and 4-Hydroxyphenylacetic acid	Root exudates	Seal <i>et al.</i> (2004b)
	5-Hydroxyindole-3-acetic acid	Root exudates	Seal <i>et al.</i> (2004b)
	4-Phenylbutyric acid	Root exudates	Seal <i>et al.</i> (2004b)
	Abietic acid	Root exudates	Seal <i>et al.</i> (2004a)
	Lanast-7,9(11)-dien-3 α ,15 α -diol-3 α -D-glucopyranoside	Rice hull extract	Chung <i>et al.</i> (2006a)
	β -Resorcylic acid	Straw	Kuwatsuka & Shindo (1973)
	Resorcinols	Root exudates	Bouillant <i>et al.</i> (1994)

However scientific research on the allelopathic effects of rice varieties is still scarce and at the same time rice is being the most important food crop in the world has the possibility to take the lead in this new

scientific challenge. The investigation of allelopathy shows that it is promising to use allelopathic activity against weed in sustainable agriculture systems. Rice allelopathy has been confirmed as an inducible genetic trait (Bi et al., 2007) that is associated with molecular regulation of secondary metabolic pathways (Bi et al., 2007; Song et al., 2008; Fang et al., 2009). Therefore, it might be feasible to trigger allelopathic potential through alteration of agricultural practice or manipulation of environmental conditions. Further study needs to be directed to this end to elucidate the molecular genetics and enzymology of the metabolic pathways associated with the biosynthesis and liberation of allelochemicals, and to establish a series of specific management strategies that can be applied to crop production to avoid down-regulation of the relevant genes associated with allelopathic activity (Belz, 2007; Macías et al., 2007; Fang et al., 2009). Moreover, to achieve agriculture based on low inputs of products released into the environment, the use of allelopathy must be complemented by other techniques that contain the development of undesirable species, such as bioherbicides and soil tillage methods, requiring careful integration of all these (Masum et al. 2015). Rice allelopathy is not only determined by plant-to-plant interaction, but also involves chemical interventions mediated by the soil, which can be regulated by various environmental factors, so much so that these effects are considered key methods for improving its effectiveness. Studies have shown that habitat changes could influence the synthesis and type of allelochemicals released. (Gaofeng et al., 2018). Allelopathy also offers an attractive natural option to reduce nitrification and improve nitrogen use efficiency in agricultural systems. This process can occur by incorporating crop residues into the soil, with plant roots releasing allelochemicals capable of inhibiting the activity of various vital enzymes (Fig. 5.1).

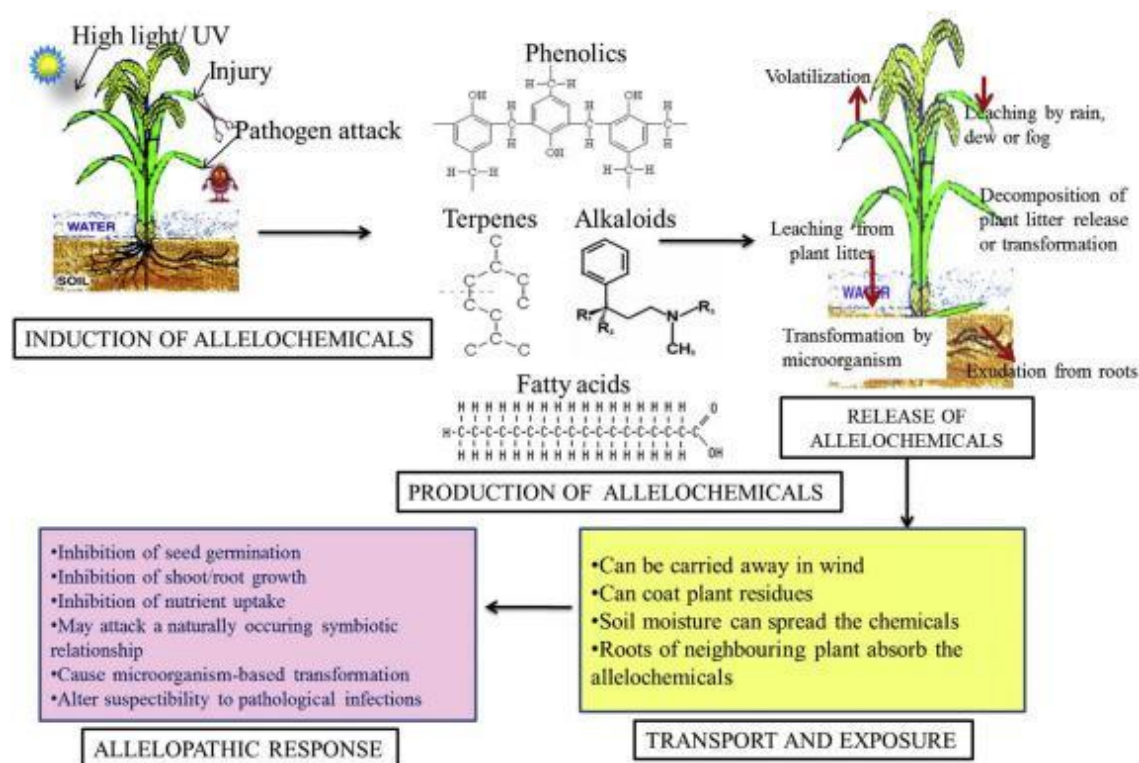


Fig. 5.1: The induction, release, transport, and exposure of allelochemicals and their presumed effects.

This review could give a great contribution on that scientific scenario to clarify and categorize the current possibilities for scaling up allelopathic rice varieties to give a new option for sustainable weed management and an alternative to conventional agrochemicals use. The innovation that we suggest, because it is still missing in literature, is to promote studies on allelopathic rice including: i) more field studies, ii) investigation on the role of biodiversity (e.g. fungi and bacteria) involved in the allelopathic compounds convey from rice plants to soil and seeds or roots of weeds because previous work did not considers allelopathy and allelobiosis that are two probably inseparable processes occurring in plant–plant chemical interactions (Chui-Hua Kong et al 2024) also between rice and weeds during the growing season.

5.1.1 Rice Growth and the Struggle Against Spontaneous Flora in Rice Fields

Rice grown in Italy has a seasonal cycle, where it is sown in spring and matures, depending on the variety, between September and October. During its growth process, the plant reaches a height that generally ranges between 80 and 150 cm and takes about 5 months on average to fully mature. The plant grows from the seed, also called a grain or caryopsis, which constitutes the fruit of this plant species.

The rice seed is an embryo and contains all the minerals the plant needs in its early development stage. Once planted, the seed absorbs only the water needed for it to swell from the soil. The seed germination proceeds through the following phases: a) swelling of the caryopsis; b) breaking of the outer coating, emergence of the coleoptile tip, emergence of the mesocotyl, and development of the first cylindrical leaf; c) formation of the primary root, simultaneous with the elongation of the coleoptile, and formation of secondary roots. In aerobic conditions, the root system develops faster than the aerial system; the opposite occurs in submerged conditions. Until the first leaves form, the germling lives autonomously; afterward, the seedling grows by drawing from the soil. At temperatures close to 0°C, the seed swells without initiating the formation of different vegetative organs.

In summer, the plant produces an inflorescence, called a panicle (often incorrectly referred to as an ear). It can typically contain 100-150 spikelets, each topped with a flower, which blooms about 90 days after seed germination. Finally, each flower turns into a seed. (www.risoitaliano.eu, www.enterisi.it, AA.VV. (2008).

All these phases, from the growth of the plant to its maturity and readiness for harvest, are accompanied by mechanisms to protect the plant from potential diseases and the presence of weeds, which can create serious obstacles to optimal production. The presence of these factors significantly affects production through direct damage, such as yield reductions due to competition between the host population and unwanted species for space, light, water, and nutrients, or through parasitism. Indirect damage can also

degrade product quality, reducing the healthiness of the crops, which lowers their price and increases protection costs.

If no defence mechanisms were applied, 83% of the world's rice production would be lost. According to FAO data, the idea of agriculture without the use of pesticides is unfeasible because the land area needed to meet the growing demand for food, driven by population growth, would have to increase. To achieve this, hectares of prairies and forests would have to be sacrificed, causing further environmental damage. ("Growing food for nine billion", www.fao.org).

The main methods of weed control in rice paddies, adapted to the unique conditions of this environment, include indirect interventions such as soil tillage, which affects the seed bank distribution along the soil profile, as well as pests and pathogens. Crop rotation over the years is also practiced to diversify and reduce the aggressiveness of the weed flora. Direct control methods for these species mainly involve the use of chemical means, the so-called plant protection products or pesticides, which rely on molecules toxic for weeds, applied to the soil or the plants themselves. In recent decades, rice cultivation has been characterized by extensive use of synthetic herbicides, which has led to numerous environmental issues, as well as the emergence of resistance to these products. Consequently, there has been a progressive reduction in the use of herbicides for weed control, limiting the available mechanisms of action. Additionally, the prolonged and repeated use of certain herbicides with the same mode of action has exerted strong selection pressure on weeds, resulting in populations resistant to these herbicides.

In Northern Italy's rice-growing areas, rice paddies are affected by populations of weeds from three main plant families: Poaceae, Cyperaceae, and Alismataceae. The first family includes barnyard grass (*Echinochloa crus-galli*), a species that has developed cross-resistance to various molecules over the years and can survive herbicide doses typically used for its control. This plant is highly harmful due to its high invasive capacity and upright growth habit.

Among the Cyperaceae, *Cyperus difformis* (small-flower umbrella sedge) and *Schoenoplectiella mucronata* (rice-field bulrush) are of particular importance for rice crops. They exhibit resistance or reduced sensitivity to several herbicides.

As for the Alismataceae, *Alisma plantago-aquatica* is a typical aquatic weed whose presence in rice paddies is linked to the use of water-seeding techniques. These species are joined by *Heteranthera limosa* and *Heteranthera reniformis*, two exotic species that have significantly expanded their range in recent years and for which effective herbicides are lacking. Another weed capable of causing significant crop losses is *Oryza sativa* var. *sylvestica* (weedy rice), which has been on the rise. This species is distinguished by its high seed shattering, a phenomenon known as "crodataura," which allows for widespread dissemination. It resembles cultivated rice but can be distinguished by height. To combat it, a technique called "false seeding" is used, which involves preparing the soil for sowing without actually planting, allowing this species to germinate and then be mechanically or chemically removed. ("Infestanti della coltura,"

www.enterisi.it). Weed management in rice fields is crucial to ensure adequate production levels (Orlando et al., 2019, and www.enterisi.it). Studies confirm that weeds compete strongly with crops for space, light, water, and nutrients. This is exacerbated by the fact that spontaneous plants tend to grow and develop faster than rice, compromising the final yield if not adequately controlled (Dass et al., 2017). Weeds in rice paddies are specialized for this unique cultivation environment. Weed management is complex and requires a good understanding of botanical species. Weed control is carried out through soil tillage, agrotechnical methods for weed removal or suppression, and the use of plant protection products that are selective for the crop (VV.AA. 2008). In this study, the characteristics of some allelopathic rice varieties that can help in this context of "crop protection" were examined. The use of plant protection products in rice paddies is particularly studied and important due to the environmental impacts related to the water-soil-air compartments (Falzarano, 2020). In fact, when it comes to the availability of pesticides, rice farming has seen a progressive reduction in recent years, with fewer plant protection products available for weed control and a reduced number of mechanisms of action available (www.enterisi.it).

Additionally, the prolonged and repeated use of certain herbicides with the same mode of action has exerted strong selection pressure on weeds, selecting herbicide-resistant weed populations (VV.AA. 2008). Various resistant weed populations have been collected and studied, such as barnyard grass (*Echinochloa crus-galli*) among grasses (resistant to ALS-inhibiting and ACCase-inhibiting herbicides), *Cyperus difformis* and *Schoenoplectiella mucronata* among sedges (resistant to ALS-inhibiting herbicides), and *Alisma plantago-aquatica* among Alismataceae (resistant to ALS-inhibiting herbicides) (CNR, 2016).

5.2 Materials and methods

5.2.1 The selection and characterization of allelopathic rice varieties

To carry out the study and evaluation of allelopathic rice varieties, 18 accessions were selected, which had previously been the subject of research by universities in various parts of the world. Through distinct trials, their allelopathic characteristics were affirmed, and much other information was gathered, such as the substances they release, their responses to environmental changes, and the effects on neighboring plants.

The studies considered, totaling 30 papers, each conducted in a different way but often aimed at the same final goals regarding the interpretation of rice's allelopathic traits, were drawn from publications released by agricultural journals and online sites. Most of the literature used for this project comes from work done by universities located in Asia, particularly in countries like China, India, Japan, Thailand,

Bangladesh, Malaysia, South Korea, and others. This highlights the vital importance of rice cultivation in Asia, which serves as the main livelihood for a huge number of people and provides a significant percentage of daily caloric intake. However, researchers from European countries such as Italy, France, Denmark, as well as from the United States and Australia, also contributed to some of these studies. After reviewing the numerous varieties investigated in these studies, those of potential interest for Italian rice cultivation were selected, given that the field trials described were conducted in regions with climates that are often very different from those typical of Italy, and thus some varieties might not be adaptable. Out of over 100 varieties and cultivars mentioned in the referenced literature, 18 accessions were chosen to be evaluated for their characteristics. The seeds were requested from germplasm banks, which supplied them to the University of Milan. Germplasm banks are seed banks where seeds of plants considered of particular interest or threatened with extinction are cataloged and preserved. These reserve deposits play an important role in safeguarding and preserving plant biodiversity, which is fundamental for agriculture and the environment. Seeds stored in germplasm banks are kept in optimal temperature and humidity conditions to maintain their viability over time. This way, if a plant species becomes extinct, it can be recovered and reproduced. Most of the seeds, from 14 of the 18 selected varieties, were sent by IRRI, the International Rice Research Institute, an NGO founded in 1960 that conducts research and training in agriculture, headquartered in the Philippines. Its mission is to work towards eliminating poverty and hunger among people and populations who rely on rice-based agri-food systems. It also aims to improve the health and well-being of farmers and consumers, promote environmental sustainability in a world challenged by climate change, and support the empowerment of women and youth in the rice industry. The other three varieties used for the project come from the United States, specifically from the National Plant Germplasm System (NPGS), a network of institutions and agencies (federal, state, and private) dedicated to safeguarding the genetic diversity of plants important for agriculture. NPGS is managed by the Agricultural Research Service (ARS), the research arm of the U.S. Department of Agriculture, with many of its genetic banks located at state-funded university sites. NPGS's mission is to support agricultural production by acquiring and conserving crop germplasm, evaluating and characterizing germplasm, documenting it, and distributing it. As for the seeds of the last variety selected for study and evaluation, they were provided by a germplasm bank located in Côte d'Ivoire, the country of origin of this particular rice.

Once the seeds of the desired accessions were obtained, they were cataloged, and some characteristics of each variety were described. Each variety was given a proper name and a code that identifies its registration number (accession number). Additionally, the germplasm bank from which the seeds were supplied and their country of origin were recorded. (Table 5.2)

Tab. 5.2: Selected accessions and their characteristics

VARIETY	CODE	GERMPLASMS BANK	ORIGIN
AC 1423	IRGC 10636	IRRI	India
Amaroo	IRGC 82290	IRRI	Australia
Basmati	IRGC 133790	IRRI	India
Boteswar	IRGC 26579	IRRI	Bangladesh
Niamat	PI574677	NPGS	Bangladesh
Dular	IRGC 121331	IRRI	India
Giza	IRGC 10169	IRRI	India
Hinohikari	PI636852	NPGS	Japan
IAC 120	IRGC 22712	IRRI	Brazil
IAC 164	IRGC 55860	IRRI	Brazil
IAC 47	IRGC 19643	IRRI	Brazil
Oryza Glaberrima (IG1)	IRGC 102948	IRRI	Mali
Oryza Glaberrima		Africa Rice Center (Africa Rice)	Ivory Coast
Italpatna	IRGC 50336	IRRI	Italy
Oryza Glomaepatula	IRGC 82033	IRRI	Brazil
Oryza Longistaminata	IRGC 81951	IRRI	Zambia
Toro	IRGC 140	IRRI	USA
Kyeema	PI597032	NPGS	Australia

Regarding the motivations behind the selection of individual accessions for the project, these highlight the presence of specific attributes already mentioned in the publications used as references. Therefore, the accessions cited in the papers were selected precisely because of their known and observed allelopathic characteristics.

For example, the indica cultivar AC 1423 was considered because prior studies indicated its strong allelopathic potential against *Echinochloa crus-galli*, showing effects in reducing the length of their roots, shoots, and consequently, their biomass.

Another study, which involved the analysis of 27 different varieties sourced from the most important germplasm banks, IRRI and NPGS, as well as one in Australia at Yanco, New South Wales, evaluated the interaction between allelopathic rice and five rice field weeds. This was done by comparing parameters

obtained for each weed and subsequently classifying the cultivars based on their weed-inhibiting ability. Finally, a ranking was created, based on the average inhibitory effects on all the weeds. The selected varieties for this project, in descending order of inhibition strength, were: Toro, Dular, Basmati, Kyeema, Italpatna, Amaroo, and Giza.

The seeds of *Boteswar* were selected because this cultivar has the highest allelopathic potential among the 50 native rice varieties of Bangladesh, showing a strong inhibitory effect on barnyard grass (*E. crus-galli*) and other species of *Echinochloa*.

The variety named *Niamat*, or *BR21*, demonstrated a high percentage reduction in weed shoot length, as well as a significant reduction in the height of *E. crus-galli*.

Seeds of *Hinobikari*, a japonica rice variety from Japan, were chosen based on studies focusing on the contribution of momilactone B and momilactone A to rice allelopathy.

The three Brazilian cultivars *LAC 120*, *LAC 164*, and *LAC 47* also showed strong inhibitory power against target weeds, particularly under specific laboratory conditions.

Studies on *Oryza longistaminata* examined its allelopathic responses to variations in temperature and light, as this variety has accessions that are highly allelopathic.

Oryza glumaepatula, on the other hand, was tested on *Lactuca sativa* (lettuce) and inhibited root growth through the release of water-soluble allelochemicals.

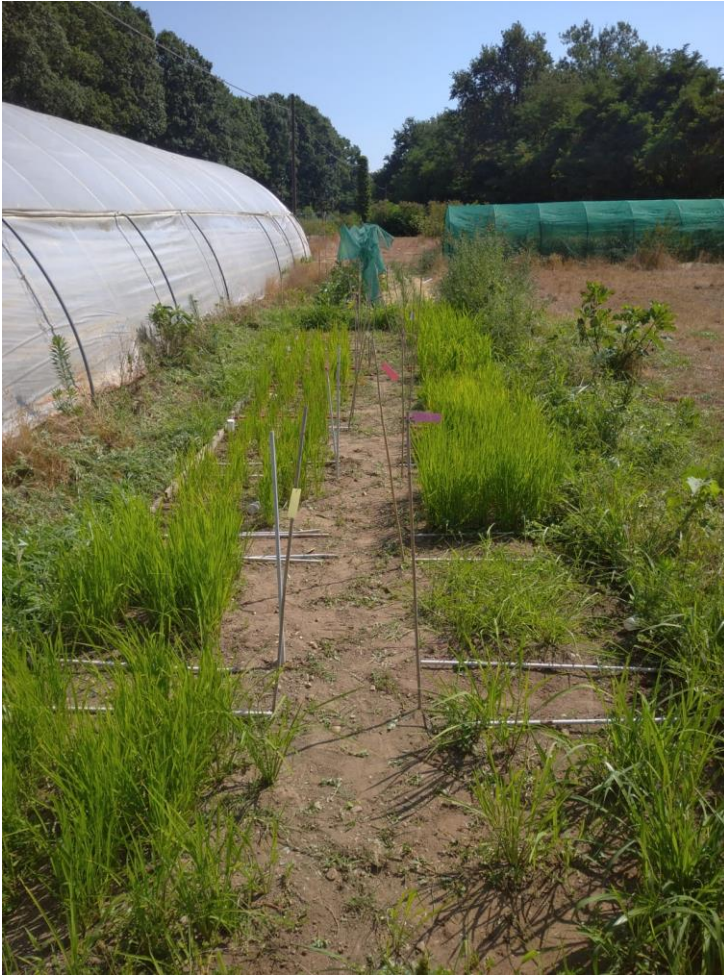
Lastly, two varieties of *Oryza glaberrima* (named *O. glaberrima* and *Oryza glaberrima IG1*) exhibited traits such as notable height, a wide germination angle, and reduced vitality of neighboring weed roots.

5.2.2 Seed replication

During the 2023 season, experimental plots of one square meter each were set up with the goal of replicating the seeds of 18 different varieties (Figure 5.1). This project was conducted at a rice farm located in Bereguardo (PV). The main objective was twofold: on one hand, to obtain a sufficient amount of seed for future studies and experiments; on the other hand, to observe which of these varieties were able to complete their life cycle in a local climate like that of Bereguardo. The information gathered will allow the selection of the most suitable varieties to be replanted in subsequent seasons, thus optimizing future research and cultivation.

An experiment of this nature is crucial for identifying the rice varieties that are most resilient under specific climatic conditions, thereby improving adaptability in similar contexts.

Figure 5.1: plots for seed replication



Of these 18 varieties, all 14 *O. sativa* varieties successfully completed the cycle up to seed reproduction, while *IG1* (*O. glaberrima*), *IRGC1* (*O. glaberrima*), *IRGC2* (*O. glumaepatula*), and *IRGC3* (*O. longistaminata*) did not produce seeds and therefore will not be carried forward for future field studies.

5.3 Results

The results focus on the analysis of 30 articles divided in 4 reviews and 27 article papers that quote directly names of allelopathic rice varieties. Articles which were discarded were: too generalist, not focusing mainly on rice, too specific for genetics or chemistry sectors and away from proposing agronomical solution.

Table 5.3 describe the articles where rice variety tested are suggested by the authors as source of genes in rice breeding program to develop high yielding and weed suppressive rice varieties. In the table we left only the rice variety/line which resulted the most allelopathic from the studies which are not citing the specific allelochemical compounds involved in the process of suppressing the target plant. While in this article the variety cited as not allelopathic were: N28S, N9S, N2S, N13S, Peiai64S, Tehuazhan35, Rexmont, Lemont. Except from the non-allelopathic variety/line cited and Jungle rice, that as not

commercial interest, all the varieties/lines of rice in table 5.3 are the most promising suggested to farmers for cultivation especially where barnyard grass is a problem.

Table 5.3: articles where rice variety tested are suggested by the authors as source of genes in rice breeding program to develop high yielding and weed suppressive rice varieties

Most Allelopathic variety/line emerged	Plant tested	Country	Year of publication	Tests place	References
BR23, BR21, BR25, BR 11, BR-5331-93-2-8-4, BR-5620-9-1-2, Lalparija, BR-5615-9-1-2, BRRI dhan27	Lactuca sativa, Echinochloa crus-galli	Bangladesh	2012	Laboratory/Green house	4_26
WITA-12, WITA-4, Fukuhibi, Kalizira, Woo-Co, WITA-3	Echinochloa crus-galli, Cyperus difformis, Cyperus iria, Fimbristylis miliacea, weedy rice	Malaysia	2018	Laboratory	8
Tsushima-akamai, Souja-akamai, Koshihikari	Lactuca sativa, Trifolium repens	Japan	2015	Laboratory	17
N2S, P1312777, No 1	Echinochloa crus-galli	Cina	2008	Laboratory/Field	19
Quest, M 205	Lolium rigidum	Australia	2012	Laboratory	20
Yongyou 15, I-Kung-Pao, Yongyou 13, Zhongzao 22	Orobancha cumana Wallr., Orobancha minor Sm	Cina	2014	Laboratory	21
Govind, UPR 2962-6-2-1	Alternanthera sessilis, Echinochloa crus-galli, Echinochloa colona, Cyperus iria, Cyperus difformis	India	2012	Field	30
Jungle rice (Echinochloa colona (L.)Link)	Rice varieties KDML 105 , RD 41)	Thailand	2017	Laboratory	31
Giza, Amaroo, Takanenishiki, Ratna, Jarrah, Palmyra, IET 1444, Bala, Kaohsiung sen 2, Millin, Illabong, Paragon, Quest, Opus, Zeiziq	Sagittaria montevidensis, Sagittaria graminea, Alisma lanceolatum, Damasonium minus, Echinochloa crus-galli	Australia	2010	Laboratory	34
Vang Quat Bien, Nanh Chon, Lua Tho, Bulu Pan dark, Huong Chiem, Nang Quat Vang, Nang Quat Bien, Ble Blau, Vang Thom	Echinochloa crus-galli	Vientname giappon e	2018	Laboratory/green house/field	38
BR17	Echinochloa crus-galli	Bangladesh	2021	Laboratory	45

5.4 Discussion

Over the last 45 years, scientists have given insights into allelopathic rice research. The main results focused on developing screening methods, and many identified allelochemicals in rice plants were published. However, to effectively manage rice allelopathy for sustainable cropping systems, the authors suggested that several works should be further carried out, including (a) the development of further screening methods in greenhouses and fields more than in laboratories to evaluate the actual allelopathic potential of rice cultivars, and (b) microbial investigation, such as fungi and bacteria biodiversity, which could enhance rice allelopathic effects against weeds. Previous work has given insight into whether the allelopathic characteristics of rice can be genetically controlled and allelochemical compounds that could be interested in weed suppression ability. A recent article suggests inoculation of carrier materials from allelopathic bacteria as weeding management in direct-seeded rice; this supports the intuition of the needed future research on the intrinsic characteristics of allelopathic rice and interaction between living

organisms (e.g. bacteria root endophytes) in the rice plant, soil system and their interactions against weeds.

5.5 Conclusion

The bibliographic research for this work allowed for the evaluation of the current state of research and application of allelopathic rice varieties.

Globally, there are numerous studies on this topic, but in Italy, further investigation and experimentation are necessary to assess the adaptability of the currently available genetic material to provide practical results for European rice cultivation.

The process undertaken in this work also enabled the identification of the main species belonging to the spontaneous flora in rice fields. The creation of seed multiplication plots ensured the growth and seed production of rice accessions obtained from germplasm banks such as the IRRI, NPGS, and African Rice.

This process allowed for a preliminary evaluation of the accessions to understand their morphological characteristics and their potential adaptation and relevance for cultivation in the context of Italian rice farming. The future goal is to test these varieties in the field and take advantage of their allelopathic traits, providing an eco-friendly alternative to traditional rice cultivation methods.

Of the 18 accessions for which multiplication plots were established, those that demonstrated the greatest compatibility with the climate and growth cycles of Italian rice-growing areas were the Dular and Niamat accessions. This is because the length of their vegetative cycles, as well as various phenotypic traits they express, align with those of many accessions already established in Italian rice cultivation.

CHAPTER 6 INTEGRATED SUSTAINABILITY ASSESSMENT OF RICE FARMING PRACTICES IN THE WORST YEAR IN 150 YEARS: INSIGHTS FROM FOUR ITALIAN FARMS.

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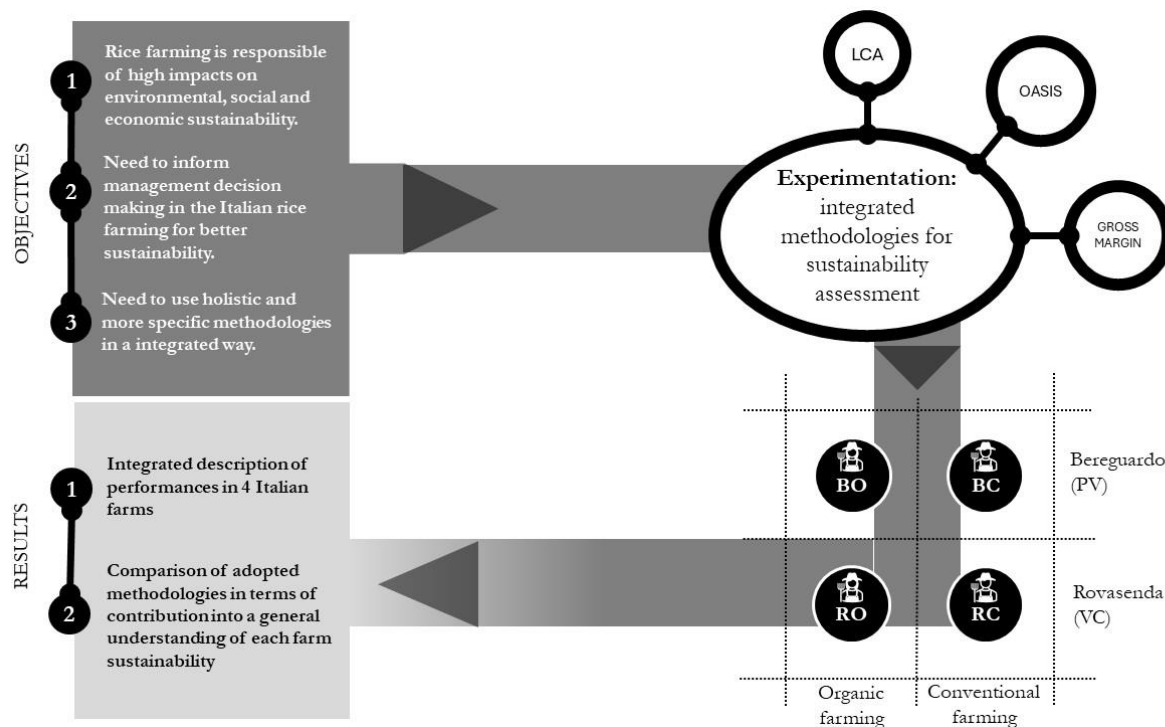
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Abstract

Integrated Sustainability Assessment of organic and conventional rice (*Oryza sativa* L.) production in four farms in Northern Italy was performed to evaluate the economic, social and environmental impacts under the extreme drought conditions experienced during 2022, the worst in 150 years (IPCC). LCA (Life Cycle Assessment) method and the gross margin analysis were used to triangulate the results of the implementation of OASIS (Original Agroecological Survey and Indicator System), an indicator system developed by Agroecology European. The inputs and outputs were collected from the farm's data recording and direct interview with farmers. Economic analysis using gross margin showed conventional farms outperforming organic ones by 25%, but this gap narrowed with OASIS, which considers additional economic factors. The LCA and OASIS methodologies also produced differing environmental outcomes, influenced by whether area or mass was the functional unit. Conventional models showed less impact per ton of produce, while organic models show potentially lower impacts per hectare, with water management being a critical factor. Organic farms scored higher in biodiversity and resilience, confirming their alignment with EU and Agenda 2030 sustainability goals. However, conventional farms also demonstrated the potential to adopt resilient measures. The results from this study provide the basis for appreciating how the triangulation of different methodologies, ranging from more specialized to holistic frames, is useful for integrated sustainability assessment in rice farming, and namely to point out and describe the existing tradeoffs between the economic, environmental and social outcomes. The study's limitations include a small sample size and the complexity of comparing geographically and technically diverse systems. Future research should expand the sample and integrate more methodologies, such as social LCA and specific biodiversity assessments, to provide a more comprehensive understanding of sustainability in farming systems.

Graphical Abstract



6.1 Introduction

With over half the world's population relying on rice as a staple, and its cultural, economic, and nutritional unmatched significance, rice cropping's role in shaping more just and sustainable food systems is widely accepted (Hashim et al. 2024; Ahmad Rizal et al. 2024; Chivenge et al. 2020). Yet, rice cropping exacts severe environmental tolls, including land degradation and greenhouse gas emissions. With rice covering approximately 167 million hectares globally, its water-intensive nature, consuming 34-43% of the world's irrigation water, exacerbates water scarcity issues. Furthermore, the flooding of paddy fields contributes to around 10% of global methane emissions, highlighting its environmental impact (Mahabubur Rahman e Yamamoto 2021).

Rice cropping's environmental and social challenges are so tough because rice cultivation persists globally, with over 715 million tons harvested annually across more than a hundred countries. China and India lead the global production, while Italy emerges as Europe's primary rice producer, with around 230,000 hectares cultivated. Italian farms predominantly cultivate japonica varieties, and witness a growing interest in sustainable agricultural practices, evidenced by the increasing adoption of organic rice cultivation. Therefore, Italian rice farming plays a significant role in Europe's sustainable food system (Arcieri e Ghinassi 2020), requiring evaluation across environmental, economic, social aspects.

The debate now insists on the model of rice production that may better contribute to matching the agrifood sector transition agenda. In fact, organic rice farming and fair trade value chains constitute an interesting alternative that is currently studied to understand its potentials for the mandatory transition of global food systems (FAO 2021; Quiédeville et al. 2018; Nuntana Udomkit e Adrian Winnett 2002).

Previous research comparing conventional and organic rice cultivation in Italy encompasses a broad spectrum of socioeconomic and environmental assessments, but focus mainly on specific dimensions, mainly on emissions, soil quality, agricultural practices, health impact and gross margin studies. Some studies focus specifically the environmental impact and the pollution generated by different cropping managements (Onorati et al. 2022; Nadimi-Goki et al. 2018; Landi et al. 2018; Nadimi-Goki et al. 2014; Bechini e Castoldi 2009). Studies using Life Cycle Assessment (LCA) on Italian rice cropping reveal significant environmental impacts (Bacenetti et al. 2016; Bocchi, Ferrero, e Porro 2007; Blengini e Busto 2009; Hatcho et al. 2012; Fusi et al. 2014). Blengini et al. conducted an LCA in Vercelli, highlighting CO₂eq emissions, energy consumption, and water use per kg of rice (Blengini e Busto 2009). Despite potential yield reductions, they advocated for organic and upland farming to mitigate these impacts. Bacenetti et al. (2016) assessed organic rice cultivation in Lombardy, identifying methane and nitrogen emissions, and mechanization as key concerns. Vaglia et al. (2022) emphasized the environmental and health challenges of rice farming in Italy, advocating for organic agriculture to align with sustainable food production goals, highlighting reduced environmental impacts of organic methods (Vaglia et al. 2022).

LCAs typically focus on biomass production for food, fiber, or bioenergy (Clark e Tilman 2017), but often overlook broader agroecological functions and ecosystem services (Huang et al. 2015). Current LCAs may misrepresent less intensive practices like organic agriculture (Van Der Werf, Knudsen, e Cederberg 2020; Winter et al. 2017; European Commission 2010), inadequately addressing biodiversity, pesticide effects, animal welfare, and nutritional quality (Van Der Werf, Knudsen, e Cederberg 2020; Winter et al. 2017; European Commission 2010). Integrating LCA with broader sustainability assessments could enhance decision-making for rice cropping (Romani M. e Beltrare G. 2007; Hokazono e Hayashi 2012). Few assessments in rice production focused specifically on the socio-economic dimension (Vinci et al. 2023). Social impacts of rice cropping have been studied mainly in connection with fair trade initiatives, looking at the extent to which imported rice affect the social sustainability of the European food consumption (Mancini et al. 2023). Some previous papers analyze the economic sustainability of production systems in rice cropping, confirming a tradeoff between economic impact and socio-environmental impacts exist in the Italian context (Castoldi e Bechini 2010; Bechini e Castoldi 2009; Tesio et al. 2014). From the economic standpoint, the gross margin method is a widely recognized indicator to provide clear and immediate evaluation of agricultural activities in terms of financial returns, especially useful for comparisons between conventional and agroecological cropping models (Boval, Bellon, e Alexandre 2014; Benoit et al. 2023; Virginia et al. 2018)

All these studies collectively enrich understanding of rice cultivation in Italy, providing crucial insights into environmental sustainability, soil management, and the potential of organic and hybrid farming to improve productivity while mitigating environmental and social impacts.

Nevertheless, from an agroecological perspective, social, environmental and economic impacts of the management choice are equally important and consequently it is fundamental to understand the tradeoffs between the impacts of different farming approaches, such as the conventional and the organic.

Up to our knowledge few studies address the integrated sustainability assessment of rice farming in the European and namely Italian context. Previous studies highlighted the scarce availability of clear operational data for calculating integrated sustainability indicators of rice farming. The exceptions to these limitations are represented by a few methodologies developed under the umbrella of important international organizations to promote sustainability and research efficiency in specific agricultural production systems, such as the SRP (sustainable rice platform) for rice, or the SAFA and TAPE methodologies (FAO) (Gharsallah, Gandolfi, e Facchi 2021).

Among the different methodologies to run a holistic assessment of farm sustainability, the Original Agroecological Survey and Indicator System (OASIS) method is gaining momentum due to the need to study the tradeoffs and differences among farms, taking into consideration the need to assess and communicate the complexity of the larger set of agricultural products, i.e. the ecosystem services other than food provision. As a holistic framework of assessment OASIS is based on scores defined by direct survey of primary and secondary, qualitative and quantitative, data. The OASIS tool is therefore open to be integrated and supported by other methodologies that may deepen some of the dimensions of sustainability.

To obtain a clear understanding of the tradeoffs between the economic, social and environmental dimensions among the conventional and organic model, it is necessary not only to identify production processes with the lowest social and environmental impacts, but also to identify those activities which have affordable costs while leading to higher margins, to identify sustainable management systems considering synergistically all the sustainability facets.

To this aim, and following the call for transdisciplinary evaluations of sustainability, the present research aims at: i) shedding light on the operational implementation of a mixed LCA-OASIS assessment tool, supported by a specific gross margin analysis, ii) contributing to comparing conventional and organic cropping models; iii) making this comparison in the driest year in 150 years.

6.2 Materials and Method

6.2.1 Case studies

Figure 6.1 - Geographical context of case studies. The location of the 4 farms assessed is shown on aerial photograph: near Bereguardo (PV) and Vercelli (VC).



Our research focuses on the North Italian rice cultivation district, where 4 different farms under two different production models, were monitored during 2022 cropping season. Target farms were chosen for being located in two of the most important rice-producing provinces in northern Italy, Pavia and Vercelli, and for their production model, organic or conventional. Pavia and Vercelli provinces account for 35.6% of total area and 30.1% of national production, and 32.1% of total area and 36.9% of national production, respectively (ISTAT 2022). Two farms are in Bereguardo rural area (PV), and two are in Rovasenda rural area (VC) (Figure 6.1). Located in the plains of Lombardy and Piedmont, respectively, these regions lay in a Cfa climate zone according to the Köppen classification, characterized by hot, humid summers and mild, wet winters. Bereguardo receives a mean (2021-2024) annual rainfall of 631.1 mm (450 mm in 2022, data from ARPA Lombardy), with an average yearly (2021-2024) temperature of around 13.7 °C (14.4 °C in 2022, data from ARPA Lombardy). Rovasenda, on the other hand, has a mean annual (2021-2024) rainfall of 1143 mm (576 mm in 2022, ARPA Piedmont), with an average annual (2022-2024) temperature of 13.5 °C (14.1 °C in 2022, ARPA Piedmont). These favourable climatic and hydrological conditions, coupled with rich alluvial soil composition, provide optimal conditions for rice cultivation, which has been supported over time by the development of advanced irrigation systems, including traditional water management techniques refined over centuries. The four target farms differ in terms of cropping management, with two farms implementing organic rice cropping practices (Rovasenda Organic - RO and Bereguardo Organic – BO) and two farms adopting conventional

rice farming practices (Rovasenda Conventional – RC and Bereguardo Conventional – BC). Appendix A Table 1, from A to D, describes the management practices implemented in the four target farms.

Regarding the internal organization of the farms, all farms have fields near the farm center (500 to 2000 m distance). Regarding the state of the farms in terms of mechanization, all four farms own more than one tractor and all the needed operating machines, with the only exception of one farm that relies on contractors for mowing-baling (cover crops) and harvesting (rice grains).

6.2.2 Organic model

Organic rice farming in Northern Italy employs innovative strategies to cultivate rice sustainably, focusing on weed management. These methods include mechanical weeding in dry or wet conditions and green mulching with cover crops. These methods aim to reduce reliance on chemicals, promote biodiversity, and enhance soil health. Despite challenges like precise water management and labor intensity, organic rice farming offers environmental benefits and aligns with the agroecological vision of many farmers. Three main strategies are used: mechanical weeding in dry conditions, which involves false sowing and seed drilling, and mechanical weeding in water, which combines false sowing with minimal water disturbance and seed broadcasting. Green mulching, which involves planting a cover crop before rice sowing, suppresses weeds through competition, mulching, allelopathy, and the toxicity of fermented organic matter.

In our case study, the two farms (BO and RO) followed two different models: one green mulching and the other mechanical weeding. In Rovasenda, *Lolium multiflorum* (50 kg/ha) was sown in autumn. At the end of April, water was introduced, and rice was broadcast-seeded directly onto the cover crop to exploit the allelopathic effect against weeds. In the second farm, in Bereguardo, false sowing was carried out with early flooding 15 days in advance, followed by mechanical weeding with a harrow to allow for broadcast seeding on a weed-free paddy field. Subsequently, at the end of the cycle, during the rice maturation, a pruning machine was used to remove barnyard grass and weedy rice that had grown during the season and at that stage was taller than the rice itself., and Appendix A Table 1 (A and B) describe in detail the adopted practices.

6.2.3 Conventional model

The conventional rice farming model in Northern Italy uses two main approaches: water and dry seeding. Water seeding offers thermal flywheel effects, nutritional benefits, and reduced nitrate pollution but

increases greenhouse gas emissions. Dry seeding saves water and labor but faces challenges in weed management and water management practices. Critical operations for water seeding include plowing, leveling, embankment breaking, harrowing, submersion, puddling, pre-seeding weeding, broadcasting seeding, fertilization, post-seeding weeding, harvesting, transportation, and drying. Dry seeding uses row seeding and post-sowing rolling for optimal germination. Water management concludes with pre-harvest drying for expedited harvesting and grain quality. Advanced technology in conventional rice farming reduces fuel consumption and operation time compared to organic methods.

In our case study, the two farms (BC and RC) followed two different models: in Rovasenda, water seeding was carried out, preceded by base fertilization and pre-seeding weeding 15 days prior, followed by post-emergence weeding and fertilization. In Bereguardo, dry-seeding was carried out immediately followed by pre-emergence weeding, post-emergence weeding, and two fertilizations. Our research focuses on two conventional farms that adopt both dry and wet seeding methods, and Appendix A Table 2 (A and D) describe the adopted practices in detail.

For all the farms, information concerning the agricultural practices, agronomic inputs, and the resulting grain yield (ton/ha at 14 % moisture), reported in Appendix A (Table 1 and 2), were collected through face-to-face farmer interviews and field surveys, during the 2022 cropping season.

6.2.4 Mixed methods approach for integrated sustainability assessment

Sustainability assessment of agricultural farms requires a holistic approach to capture relevant tradeoffs between the economic, social and environmental impacts of the activities. Mixed methods are extremely useful when dealing with multifaceted concepts such as sustainability because they allow to consider separately the facets of each sustainability dimension, with an adaptable degree of detail, and then to triangulate the results to build solid knowledge of the tradeoffs existing between sustainability dimensions. Our research adopts a mixed method that integrates an LCA analysis and a Gross Margin economic analysis in a widely accepted, holistic framework for agroecological performance assessment, i.e. the Original Agroecological Survey and Indicator System - OASIS, in different Italian areas. The overall objective is to provide a comparative tool for strengthening the evidence about organic model impacts, when compared with conventional production model, in dry cropping season such as 2022 in Northern Italy.

6.3 Original Agroecological Survey Indicator System

The Original Agroecological Survey Indicator System (OASIS) is a comprehensive framework designed to assess farms' agroecological transition (Peeters et al., 2021). It combines rigorous data collection through interviews, systematic transcription and calculation, and comprehensive reporting to evaluate farm practices holistically.

OASIS includes a comprehensive set of criteria and indicators divided into various dimensions such as agroecological farming practices, economic viability, and socio-political aspects. Specific indicators and scales support each criterion to measure different aspects of farm performance.

According to the methodology, we followed a three-step procedure.

During step 1, i.e. Study, an in-depth study of the OASIS evaluation criteria was conducted. All indicators proposed by the OASIS operational manual (Škorjanc et al. 2021) were selected for us in the interview. Each criterion was operationalized with definitions, best practice examples, suggested indicators for evaluation, corresponding interview questions, and interpretation scales consistent with the local context. For specific criteria, such as variable and fixed costs, we used separate indicators requiring specific information like quantities of inputs or expenditures, for other criteria we relied on the scoring provided by the interviewed farmer.

During step 2, i.e. Interview, data were collected through direct interviews with the four farmers, their families and workers. According to the OASIS guidelines, each block of questions in the survey form is linked to specific criteria to aid in coding interviews. Surveyors were encouraged to be observant and attentive, allowing for flexibility in the interview to cover additional relevant topics as needed. This ensured a thorough and holistic collection of data.

The interviews were conducted during the winter of 2022. Before starting the interview, each farm was requested to bring the farm map with crop distribution, records of pesticide, fertilizer, and BCA (biological control agents) application, and annual expense records in order to obtain the most objective and truthful data possible.

During the interview, more than 250 questions are asked, which can be summarized into five main groups (Table 6.1): an initial phase of descriptive questions about the farm, a section regarding crops and their management, a third phase concerning costs and revenues, a fourth focusing on life quality and gender equity, and finally, the last phase on labor conditions.

Table 6.1 - Structure of OASIS survey form. The table shows the number of questions for each section of the OASIS survey form.

Subject	Number of questions
Descriptive data about the farm	20

Crops and management	110
Costs and revenues	75
Life quality and gender equity	30
Labor conditions	25

During step 3, i.e. Transcription and Calculation, data were organized into a spreadsheet designed for this purpose. The spreadsheet includes all the necessary criteria names and corresponding scales. After inputting the data, average values are calculated, and radar charts are created using a provided template. This step ensures that the qualitative data collected during the interviews are translated into quantitative metrics that can be analyzed and compared across different farms.

6.4 Life Cycle Assessment

6.4.1 Goal and scope

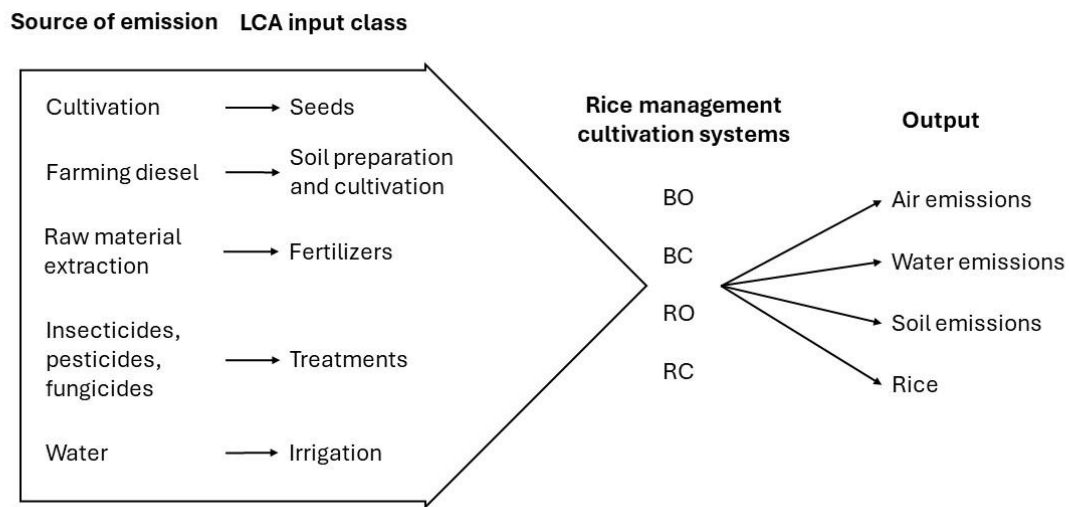
Life Cycle Assessment (LCA) methodology has been used to estimate the environmental impacts of four rice management scenarios (BO, BC, RO, RC). The standards ISO 14040/44 (ISO, 2021) and the Product Category Rule guideline “ARABLE AND VEGETABLE CROPS” developed by International EPD System were used as references (International EPD System 2023).

According to ISO standards (2021), the functional unit (FU) provides a reference to which the inputs and outputs can be related. To enable comparison of the different scenarios, the FU defined in this study was ‘the management of one hectare of rice field during the year 2022’.

A from-cradle-to-farm-gate LCA was performed. Figure 6.2 indicates the life cycle steps assessed in all the rice cultivation management scenarios.

Data regarding all agricultural operations were collected from farm diaries. All the model data was based on the Ecoinvent database (version 3.9).

Figure 6.2 - Life Cycle Assessment Workflow. The sources of emissions are listed and linked to the LCA input class, which are assessed for each scenario to produce estimations of output emissions.



6.4.2 Life Cycle Inventory

Life cycle inventory consists of a detailed compilation of all the relevant inputs (material and energy) and outputs (gaseous, liquid and solid emissions to air, water or soil) at each stage of the life cycle studied. Mainly, the data collected through agricultural diaries of farms and interviews focused on the different operations and inputs inserted in the field. Soil preparation and cultivation using agricultural machinery were modelled using the amount of fuel consumed. This was quantified for each operation as visible in Appendix A Table 6.1. Specific consumption data and related emissions were quantified for each scenario, and datasets from the Ecoinvent database were selected for these voices. The use of herbicides, fungicides and insecticides were considered in all four scenarios, and the active ingredients were used as drivers to model the different substances applied in the field. Regarding fertilizers, the nutrients that compose substances (N-P-K) and their quantities were considered and datasets from the Ecoinvent database were selected. Regarding field emissions, ammonia (NH₃), nitric oxide (NO), emissions of CH₄ from paddy water and nitrous oxide (N₂O) were quantified. Water emissions were computed considering leaching and runoff of nitrates (NO₃⁻). Furthermore, the impact on the three environmental compartments (soil, air and water) of the applied pesticides was quantified based on their active ingredient content. Quantities calculated for every emission for every scenario are reported in Table 6.2.

Emissions of ammonia (volatilized kg NH₃-N per kg of N applied) and nitric oxide (volatilized kg NO-N per kg of N applied) were calculated by multiplying each kg of applied nitrogen (N) by an emission factor, differentiated according to the type of the fertilizer (IPCC 2019). Direct and indirect emissions of nitrous oxide (N₂O) were estimated using the emission factor reported in Zampori and Pant

(European Commission. Joint Research Centre. 2019), namely: 0.022 kg of N₂O emitted to air per each kg of N synthetic fertilizer and manure applied. Direct emissions of CH₄ from paddy water were estimated using the IPCC standard. The emissions into water due to leaching and runoff of nitrates (NO₃⁻) and phosphorus (P) were calculated using the emission factors reported in Table B.16 of Zampori and Pant (2019), which are 0.24 kg of NO₃-N emitted per kg of N in fertilizers applied. Pesticide emissions were calculated by taking active ingredients into account. The approach reported by Zampori and Pant (2019) was adopted: pesticides applied in the field were assumed to be 90% emitted into the agricultural soil compartment, 9% emitted into the air and 1% emitted into water.

Table 6.2 - Emissions for each studied scenarios. BO: Bereguardo Organic, BC: Bereguardo Conventional, RO: Rovasenda Organic; RC: Rovasenda Conventional.

Emissions	Unit of measure	BO	BC	RO	RC
Ammonia volatilized	kg NH ₃ -N/ha	5,06	11,99	18,45	42,28
Nitrogen monoxide	kg NO-N/ha	0,00	5,16	0,00	0,45
Direct and indirect emissions of N ₂ O	kg of N ₂ O/ha	0,04	2,52	0,13	0,91
Direct emissions of CH ₄ from paddy water	kg of CH ₄ /ha	47,98	29,11	289,29	493,35
Nitrates leaching and runoff	kg of NO ₃ ⁻ /ha	21,09	140,62	76,88	164,13

6.4.3 Life Cycle Impact Assessment

The software Simapro version 9.5 (PRè Sustainability, Amersfoort, The Netherlands) and Ecoinvent database (version 3.9) were used to assess the environmental impacts of the four rice cultivation management scenarios considered. Among the various assessment methods available in SimaPro, we have chosen to use the Environmental Footprint Method 3.1 developed by the European Union Product Environmental Footprint. The environmental footprint (EF) method 3.1 v. has a global scope. It can be used to analyse intermediate impact categories, including characterization, normalization, and weighting factors (European Commission. Joint Research Centre. 2019). The impact category definitions, method performance, characterization and normalization adopted in this study can be consulted in the European Commission technical report (European Commission. Joint Research Centre. 2019). All 16 impact

categories of the EF method were initially used for each step of rice cultivation management (Fig. 1), and are: Acidification (ACID); Climate change (CC); Ecotoxicity, freshwater (ETX-FW); Particulate matter (PM); Eutrophication, marine (EU-MAR); Eutrophication, freshwater (EU-FW); Eutrophication, terrestrial (EU-TERR); Human toxicity, cancer (HT-C); Human toxicity, non-cancer (HT-NC); Ionizing radiation (IR); Land use (LU); Ozone depletion (OD); Photochemical ozone formation (POF); Resource use, fossils (RU-F); Resource use, minerals and metals (RU-MM); Water use (WU).

6.5 Economic analysis

The analysis of expenses associated with four case studies pertains to a single season and considers only operating costs (variable costs). Despite relying on certain assumptions and offering partial economic insights, comparing expenses and revenues across various technical and management scenarios helps evaluate trade-offs between the economic, environmental, and social impacts of different production models.

The costs of the four management models were grouped as follows: (a) Fuel consumption, (b) Water, (c) Seeds, (d) Treatments, (e) Fertilizations, (f) Depreciation of equipment, and (g) Contractors. Incomes were estimated based on the official selling prices of conventional and organic rice in the Italian Bourse (autumn 2022). Appendix A, Table 1 provides detailed information about each cost category.

Fuel costs were aggregated regardless of the operation for which the fuel was used. Diesel fuel costs were calculated based on the market price and the tax relief regime as of January 2022, using average consumption coefficients for different tractors. This estimation was based on fuel consumption per operation provided by the National Union of Agricultural Contractors (UNCAI).

Water for the four farms comes from a local network managed by the Irrigation Consortium (or similar organization). Only the resource cost was considered, assuming uniform water supply system upkeep and provision to fields without active pumping.

Seed usage ranges between 180 and 200 kg per hectare, with costs from 130 euros for the variety Baldo to 200 euros for the variety Carnaroli (per 100 kg). Sowing is performed using rotary seed spreaders across all farms, with varying tractors affecting fuel consumption and costs.

Treatments in conventional farms include commercial herbicides, fungicides, and insecticides, whereas organic farms use approved fertilizers. Appendix A, Table A lists fertilizers used in the four farms, corresponding to common rice cropping practices. Organic farms use specific fertilizers like hoof horn and cow manure, with doses varying among farms and management models (see Appendix A, Tables 6.1 to 6.4). Typical applications include diammonium phosphate at 150 kg/ha, potassium sulfate at 180 kg/ha, hoof horn at 250 kg/ha, and manure at 3000 kg/ha.

Yearly amortization per hectare of mechanical equipment was calculated based on a 15-year life span for tractors and operating machines, serving different areas across the farms (see Appendix A, Tables 6.1 to 6.4).

Some farms rely on contractors for tasks such as mowing, baling, and harvesting. Cost estimations were based on 2022 regional contractors' rates provided by the Italian Confederation of Agri-mechanics and Farmers, validated by the involved farmers, and fuel consumption per operation supplied by UNCAI.

Gross income was calculated using official stock exchange prices for conventional and organic rice, specifically the Carnaroli variety. This income was then used to calculate the gross margin by subtracting variable costs. Notably, 2022 saw the selling price of organic rice nearly equal that of conventional rice due to a general shortage that disproportionately increased prices for both. Additionally, the three varieties (Baldo, Carnaroli, and Rosa Marchetti) have different average yields per hectare. The unique price context of 2022 influenced the differential margin results between conventional and organic farms.

6.6 Results and discussion

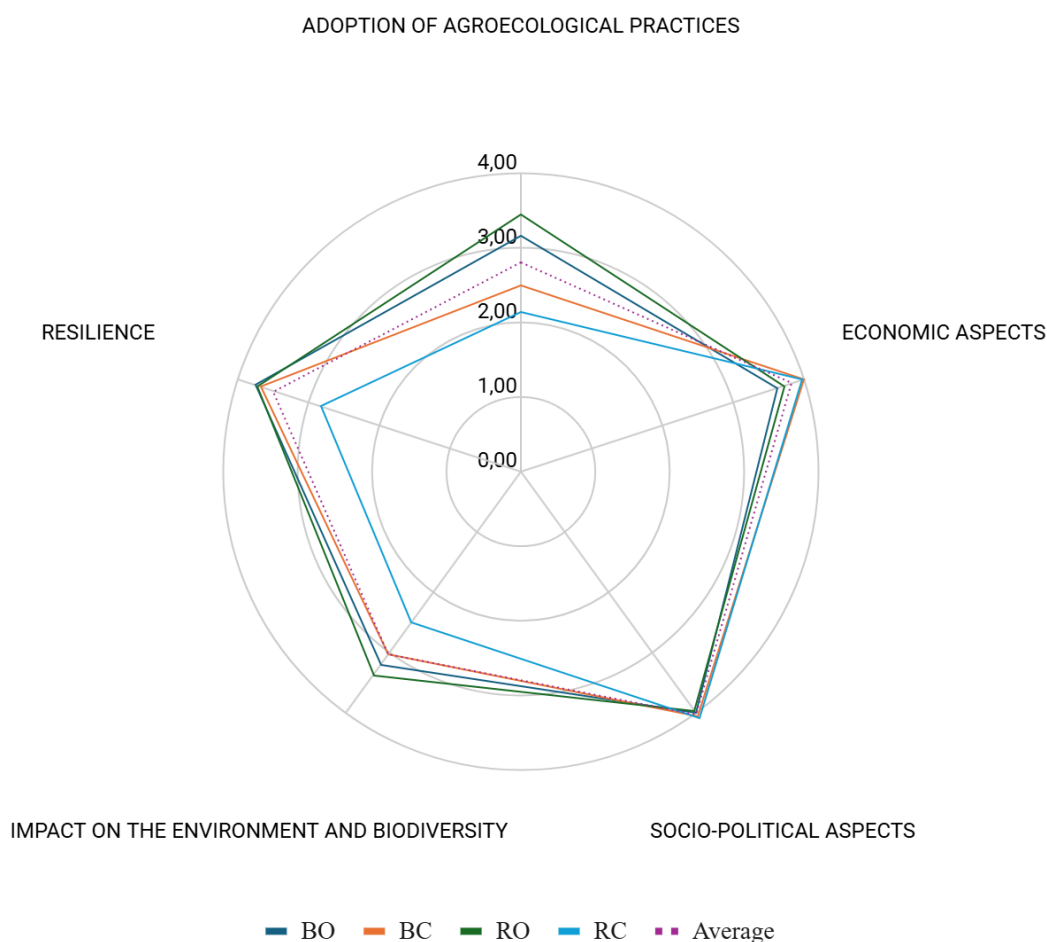
6.6.1 Sustainability assessment of case studies (OASIS)

Table 6.3 and Figure 6.3 show the overall scores for the five indicators selected to describe the agroecological performances of target farms. Appendix A Table 6.3 contains further details concerning the chosen criteria and the list of indicators used to commute the overall scores.

Table 6.3 – Results from the OASIS scoring procedure. The overall score for the five dimensions of analysis for RO: Rovasenda Organic, RC: Rovasenda Conventional, BO: Bereguardo Organic, BC: Bereguardo Conventional. A $\pm 0,2$ threshold centred on the group average score has been used to determine the extent to which each farm performs in comparison with the others.

Analysis dimension	BO	BC	RO	RC	Average	SD
ADOPTION OF AGROECOLOGICAL PRACTICES	3,15	2,50	3,45	2,15	2,81	0,59
ECONOMIC ASPECTS	3,63	4,01	3,72	3,99	3,84	0,19
SOCIO-POLITICAL ASPECTS	3,99	4,05	3,97	4,07	4,02	0,05
IMPACT ON THE ENVIRONMENT AND BIODIVERSITY	3,20	3,02	3,37	2,50	3,02	0,38
RESILIENCE	3,74	3,67	3,72	2,83	3,49	0,44

Figure 6.3 - Agroecological performances of the four case studies across the OASIS dimensions.



RO farm leads the group in adopting agroecological practices, focusing on ancient varieties and soil fertility management. It avoids pesticides and herbicides, using sulfur-based treatments and intercropping. However, RO scores lower in soil management practices and lacks a structured agroforestry system. BO follows RO closely in pest management and seeds, using 60% of seeds from the previous year and employing cover crops. BC and RC perform below average in terms of agroforestry systems and tree planting.

Economically, BO and RO are slightly below the group average, with fixed and variable costs in line with other farms. They generate income through direct sales and product processing, resulting in a short supply chain with at most one intermediary between producer and consumer. However, they do not achieve particularly positive economic outcomes relative to existing investments.

RC and BC show average performance in fixed and variable costs, with BC having the highest overall value due to above-average economic profits. RC faces high variable costs for energy use and paid labor, but its ability to transform agricultural production value into profit is notable due to local processing and diversified marketing. BC scores below average in product valorization, leading to less development of local markets and social networks.

The four case studies show similar scores in sociopolitical aspects due to the adoption of ideal human resource management, economic treatment, and care in selecting rice varieties with cultural value. RO, a farm with a keen interest in agricultural activities and the organic approach, ranks above average in cooperation in social networks and activities to respect and enhance local culture and tradition. However, RO scores below average in quality of life, with workers complaining of heavy workloads and pressure to achieve productive results. RC and BC stand out in terms of workers' contractual conditions, with RC participating in agroecology training circuits and using ancient or traditional rice varieties. RC shares a positive future perspective in farming with RO, but does not invest in cooperation with networks and associations and are less involved in local and social market participation.

From a resilience standpoint, BO, BC, and RO score equal to or above average, while RC scores significantly below. RC shows below-average values in climate resilience and farm autonomy, aligning with the average for economic resilience. However, RC struggles with efficiency and profitability compared to the average, as the need to work locally and increased demand for white rice lead to excessive work and stressful conditions.

BO and RO farms have improved resilience scores due to economic and climate resilience, with a focus on diverse products and crop rotations. They also have lower dependence on external inputs. However, climate resilience is hindered by less rustic rice varieties and lack of water storage facilities. Organic farms, like RO and BO, achieve higher scores in environmental impact and biodiversity due to good soil and carbon management, substantial woodlands, and hedges. RO has a higher biodiversity impact due to diverse production. However, soil compaction lowers their environmental scores. BC achieves the highest score in environmental impact due to soil compaction, erosion, or salinization issues. RC follows BC in terms of environment and biodiversity impact but provides the worst example due to a monocultural approach and lack of trees and wetlands.

Life Cycle Analysis

Figure 6.4 - Comparative results of the four rice management scenarios (BO, BC, RO, RC) using the EF 3.1 method. LCA impact classification is used.

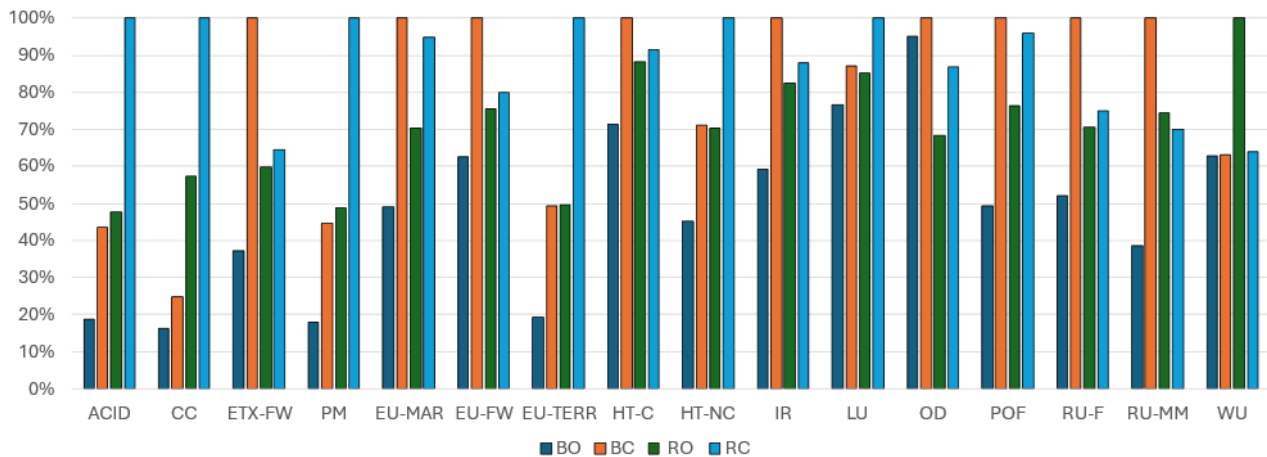


Figure 6.4 presents a comparative analysis of the four rice management scenarios (BO, BC, RO, RC) using the EF 3.1 method. The analysis is expressed as a percentage on the vertical axis, while the horizontal axis represents each impact category. For each category, the scenario with the highest impact is set at 100%, and the other scenarios are compared to this value. The supplementary data (S1) provides the numerical values for each scenario and each impact category.

The results demonstrate that the scenarios exhibit diverse environmental performance across all impact categories. Upon examination of the raw data from the comparison, an analysis of the average impact percentages in all categories indicates that RC has the highest average impact (88%), followed by BC (80%), RO (70%) and finally, BO (48%). This indicates that, in general, the RC scenario has the highest environmental impact.

However, when examining the individual scenarios and specific impact categories, the BO scenario exhibits lower impacts than the others in the majority of impact categories (15 out of 16), with the exception of OD where the best performance is represented by the scenario RO. A comparison of the four scenarios reveals an increasing trend between the organic and conventional scenarios in all impact categories. In at least one of the conventional scenarios (BC and RC), the impact levels are higher than in the organic scenario (BO and RO) apart from WU impact category where the water management for RO is almost 40% more than the conventional.

Figure 6.5 – Comparative results of gravity analysis within different scenarios.

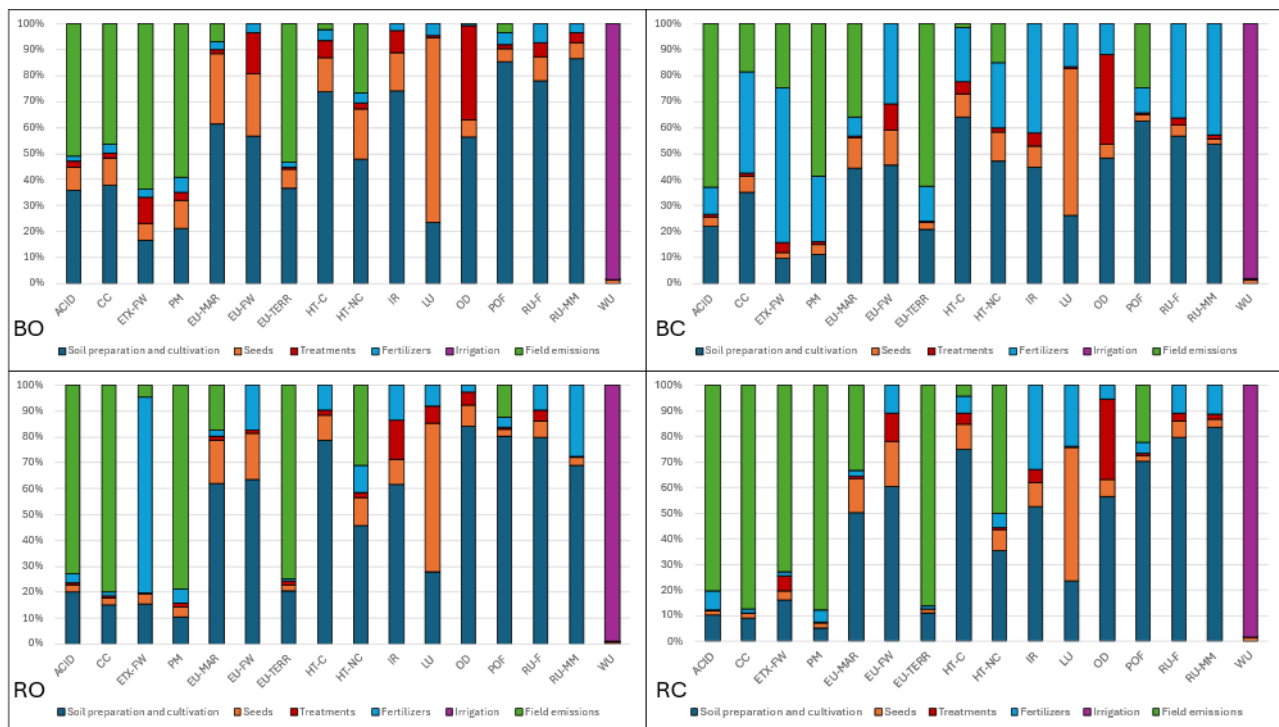
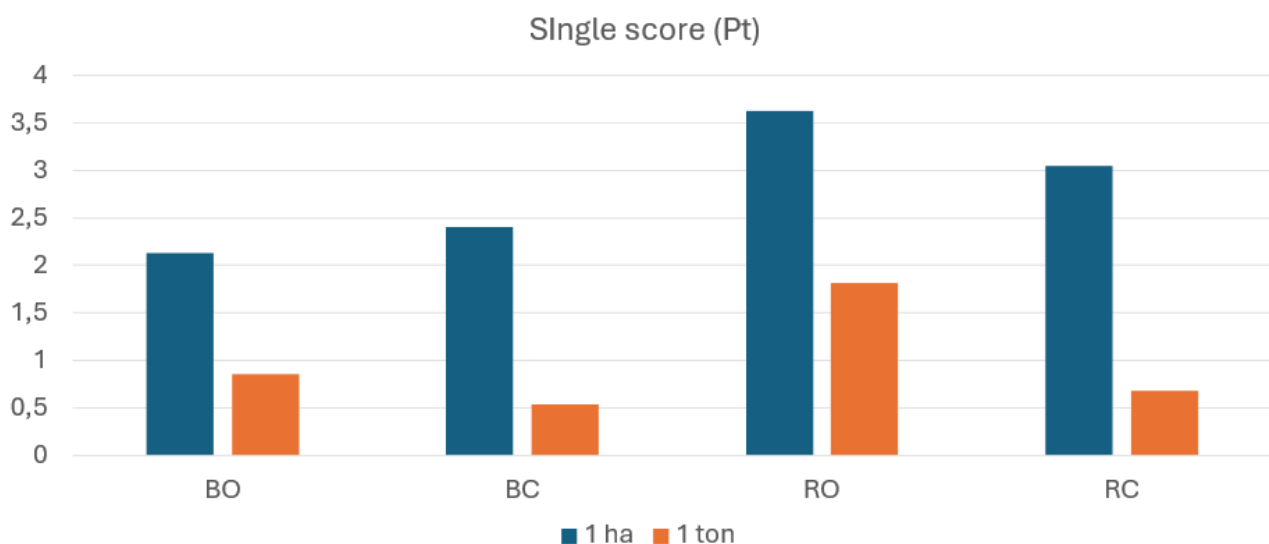


Figure 6.5 focuses on the gravity analysis within different scenarios. The supplementary data (S1) provides the numerical values for each scenario and each impact category. The analysis shows that soil preparation and cultivation activities represent the largest contributions of environmental impact (BO 49%, BC 37%, RO 46% and RC 40%). The second and third largest contributors vary depending on the scenario being considered. In the BO, RO and RC scenarios, field emissions have an average between impact categories of 20-33% and represent the second contributor. Conversely, in the BC scenario, the second contribution comes from the fertilizer production of 24%. The primary differences between scenarios for the same category of environmental impact reveal key contributing factors across ACID, CC, EU-FW, EU-TERR, LU, and WU. In the ACID and CC impact categories, field emissions are the predominant contributor in all scenarios driven by ammonia volatilization, with the highest impacts observed in scenarios RO and RC at 70-80%. Water management in both farms causes significant field emissions, and mitigation strategies should include alternate wetting and drying irrigation practices. Only in the case of CC and climate change is represented by fertilizer production with 39% of scenario BC, and the main contributor to climate change is represented by fertilizer production with 39% responsibility. In the ETX-FW impact category, the differences between organic and conventional scenarios are expressed by different factors. Major differences between BC and BO are related to fertilizers (BC shows 53 times more emissions than BO). In contrast, soil preparation and cultivation, field emissions, and seeds factors showed no significant variations. The EU-TERR impact category shows a lower trend, where the organic scenarios (BO and RO) showed half of the values than the conventional scenarios (BC and RC). Although the contributions in the WU impact category are

derived entirely from the management of the water resource to irrigate the fields and to keep them flooded for the required periods, in fact, to keep the paddy field flooded for a longer time and the number of flood cycles let the RO scenario represents the worst case.

To drive eco-design of rice cultivation management systems and reach more sustainable cultivation strategies, it is fundamental to focus on the factors analyzed. If one of the current paper aims is to compare different field management systems, yield (as suggested in the PCR) should be considered in the analysis, as different field management produces different results. Single score results can help identify the best-case scenario, also considering the maximum yield as the same CPC product (rice) is cultivated in different conditions.

Figure 5.6 – Single score results.



Analyzing figure 5.6, the environmental impact of 1 ha of field management systems (reported in blue columns) shows that Bereguardo management systems organic field management is better than conventional field management. When focusing the attention on the Rovasenda management systems, conversely, the conventional field management represents a better environmental footprint with respect to the organic field management.

However, when considering LCA results according to the FU “yield (tons/ha)”, conventional field management scenarios (BC and RC) obtain higher scores.

When the yield produced is considered as FU, conventional scenarios represent the best solution in both Rovasenda and Bereguardo farms, regardless of sustainable field management practices.

It may seem a paradox to reach a merged consideration between products and organization's sustainable field practices, but the two perspectives are essentially expression of two different standpoints, one more interested in narrowing the analysis on the sole provision of food, and the other more interested in informing a holistic knowledge about the whole set of ecosystem services provided by agricultural farms and landscapes. Both perspectives are important to satisfy the need for increased efficiency and the economic return in production processes, and to safeguard the environment and the community.

6.6.3 Gross margin analysis

The economic analysis produced the results summarized in Table 6.4, showing different economic returns for the different production models and farms. On average, the highest gross margin is for the conventional farming models, namely RC shows a gross profit (4379.42 €/ha, gross margin 74.86%) higher than BC farm (2,649.99 €/ha, gross margin 70.11%). Nevertheless, it should be underlined that the organic farming model allows the two farms to earn a gross profit of 1880.56 €/ha and 1318,01 €/ha respectively, showing a gross margin equal to 56.02% and 52.72% only.

In 2022, low rice yields in Italy led to minimal price differences between organic and conventional rice. The overall shortage raised prices for both types nearly equally, an unusual market condition. This influenced the study's results, making the gross margins between conventional and organic farming appear less distinct than usual. Consequently, the economic benefits of organic farming might seem understated compared to a typical year, where the price premium for organic rice would be more significant.

As shown in Figure 6.7, the main costs vary across the four scenarios. In general terms the consumption of diesel is the most relevant cost for all farms, while the second relevant cost is represented by seeds for the organic farms, and by treatments or equipment depreciation for conventional farms. Fertilization counts for an important share for all farms ranging from 8.2% in RC to 18.6% for BC. The irrigation consortium fee was equal for all the scenarios, and all four farms use direct surface water withdrawal without pumping so water management impacts 3.4-4.4% of the total cost across the four target farms.

In particular, in the BO scenario, the most relevant cost is represented by seed provision (33.8%), followed by diesel consumption (25.1%), contractors fee (14.8%) and equipment depreciation (9.3%). In fact, BO uses an organic-certified, expensive (200 euro/100 kg) Baldo rice variety, with a dose of 200 kg/ha, which is higher than the dose used by the other farms (from 180 to 190 kg /ha). BO is also the only farm in the sample that hires contractors, whose cost negatively impacts the gross margin obtained.

In BC the cost of fuel, seeds and treatments are comparable (23.7%, 20.7% and 22.8% respectively), followed by the cost of fertilization (18.6%) and equipment depreciation (9.8%). BC is conventional and

implemented, also in a not-ideal and dry cropping season, with three fertilizations and three treatments (see Appendix A Table a A-to-D), and uses high doses of chemical fertilizers (450 kg/ha divided into two interventions) and treatments (chemical weeding with Beyond 1,1 l/ha + Dash 1 l/ha + Marins 1,5 l/ha + Permit 0,05 kg/ha and fungicide one l/ha).

In the RO scenario, the most important cost per ha is seed provision (27.3%), followed by diesel consumption (25.0%) and equipment depreciation (21.9%). Following the organic model, fertilization counts for 15.2% and treatment only for 6.8% of the total cost per hectare.

RC scenario shows its highest share of cost for equipment depreciation (32.2%), followed by diesel consumption (23.9%), treatment and seed costs (18.8% and 13.6% respectively). In fact, RC does not use contractors for any of the farming activities, and relies on owned machineries, showing the highest number of tractors within the sample.

Figure6.7 7 - Share of variable costs for each target scenario.

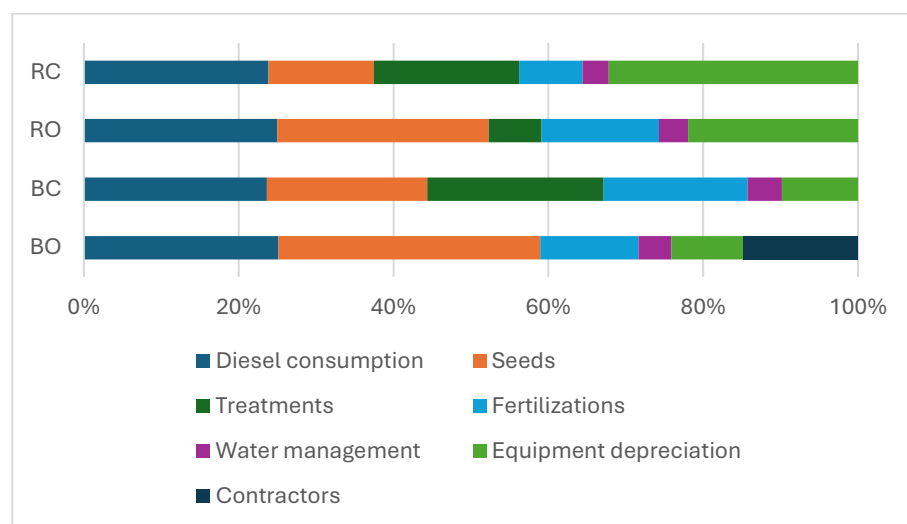


Table 6.4 - Gross margin results, and Variable costs for each target farm, and for the two considered production models. Per hectare.

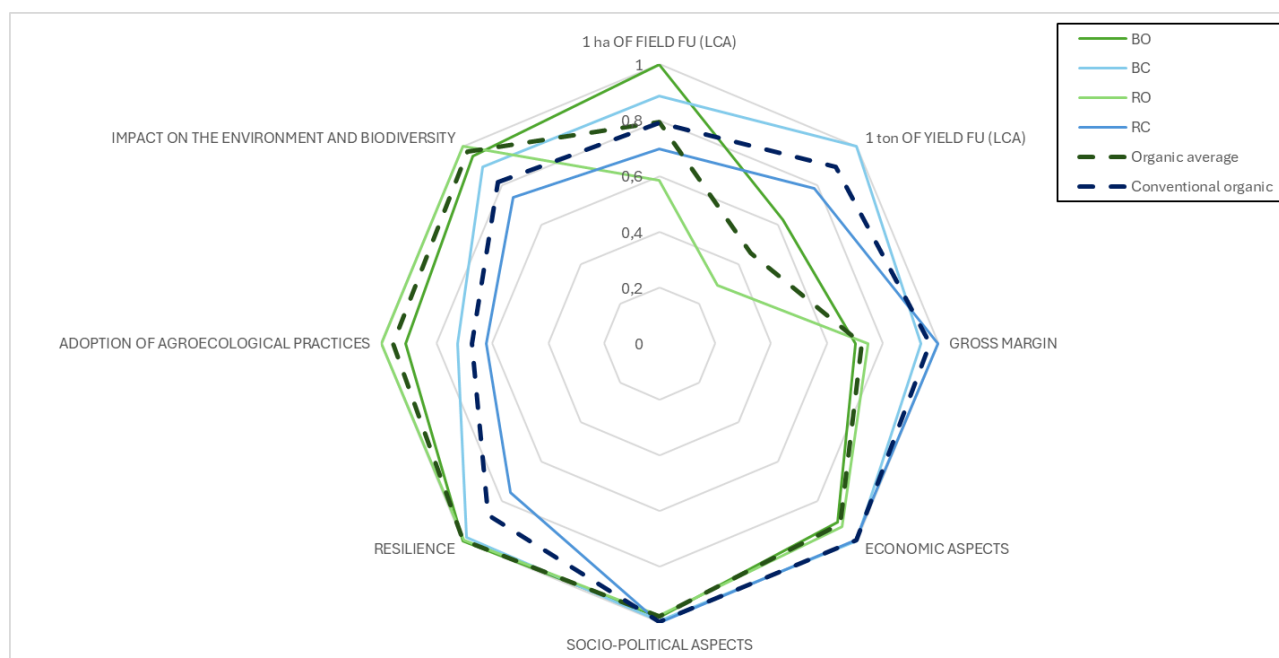
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6.6.4 Integrated sustainability assessment

The adopted mixed method allows an overall evaluation of farm sustainability by considering, comparing and cross-checking the results from different methodologies from the environmental and economic standpoint, which are indeed the most debated dimensions when dealing with the comparison of the organic and conventional farming systems. Moreover, the adoption of a holistic methodology such as OASIS include the analysis of the social dimension regarding the four target scenarios and to inform the assessment of the tradeoffs between the impacts of the studied farming models across the three main dimensions of sustainability.

Regarding the different results proposed with the three methodological approaches in the previous sections, the conventional and organic scenarios were averaged to propose a benchmark to provide an overall evaluation of the sustainability of rice cultivation in the four scenarios (Figure 6.8). The radar graph below proposes the scores obtained by each target farm rearranged and measured against the best scenario, which is different for each indicator. Value “1” indicates that the farm (or management system) reaches the best performance within the target group.

Figure 6.8 - Integrated sustainability assessment showing the results of LCA (single score), Gross margin, and OASIS assessment of the four target farms, compared to the average score obtained by the organic and conventional models.



From an economic point of view, the results of the Gross margin analysis and OASIS seem to mutually confirm the positioning between the farms and the production models. Nevertheless, differences are captured differently by the two methodologies. While from the gross margin analysis standpoint there is a clear gap of 25% in favor of conventional farms, which outperform the organic ones, the gap reduces

when considering the OASIS results. However, it must be highlighted that 2022 was a very particular year where the prices of paddy rice on the market, both for the organic and conventional have become very close. It means that normally the organic price is much higher than the conventional one, and therefore in "normal" years the gap in the two gross margins would have been decidedly smaller. Moreover, concerning comparing the two adopted methodologies the Gross margin method seems to highlight the reduction of revenue for the organic model. In contrast, the OASIS indicator about economic aspects seems to capture a less relevant difference. This difference between the results of the two methodologies is attributed to the type and diversity of data elaborated. In fact, OASIS considers other facets of the company's economic behavior in addition to the difference between variable costs and revenue. Among the aspects not considered by the Gross margin, an indicator relating to fixed costs has to be underlined. Furthermore, we can also mention the economic treatment of workers and the ways in which income is generated and transformed into profit, such as the type of market used, food processing on a farm scale, and the comparison, as a farmer's perception, with existing trends in the study area. In other words, the OASIS methodology can capture more information, even if in a more qualitative way, and to underline how the reduction of gross margin is compensated by several other differences in "how" that margin is generated.

Nevertheless, the four farms obtained very similar scores in terms of sociopolitical outcome, with one of the organic farms lowering the sub-group mean due to the massive workload entailed in organic management. The result should also be seen in the light of the context of the study, which is one of the richest agricultural areas in Italy, within one of the most profitable value chains, i.e. rice. However, OASIS is the only source of results in terms of sociopolitical dimension and no triangulation is possible with the other methodologies.

Conversely, from the environmental perspective, the LCA and OASIS methodologies produce interesting and different outcomes. Concerning the LCA comparison, and the total impact (single score) of target farms and scenarios, the results swap if we consider the functional unit of area (ha) and produce (ton). On one hand, it emerges that if one ton of product is regarded as the functional unit, the conventional model impacts significantly less as it manages to have a higher efficiency rate, i.e. input/output rate is lower. The impact is spread across several tons of production. On the other hand, if we consider the hectare as the functional unit, the result changes and the average single score for organic and conventional models overlaps. It is because BO turns out to be the farm that shows the least impact concerning the surface area, and even if RO remains the worst, it means that the organic model has potential for improvement as a whole. RO's result is mainly due to the mismanagement of water, which has a highly relevant signature in terms of GHG emissions in rice cropping (Loaiza et al. 2024; Carlson et al. 2017; Meijide et al. 2017; Dahlgreen e Parr 2024; Pathak e Wassmann 2007). In particular, even if RC and RO

show similar duration of submersion, RO adopts an additional flooding, which entails prolonged methane emissions and additional use of irrigation water. Comparing the farms on a geographical base, it should be noted that the Bereguardo farms in 2022 suffered from a nearly total lack of water, while the in Rovasenda area water was available. It entails that one possible reason for RO to implement an additional flooding was driven by the simple availability of water, against any environmental consideration. These specific features of the studied systems may explain why available literature on LCA comparisons of organic and conventional systems does not confirm the swapping (Pérez et al. 2024; Hashemi et al. 2024; Boschiero et al. 2023; Vinci et al. 2023).

It is also important to say that choosing a mass-related or area-related functional unit is widely accepted to be a fundamental decision in LCA studies focusing on comparing the organic and conventional models. According to Matthews et al. (Matthews, Matthews, and Hendrickson 2014), FU should include several dimensions which are the responses to “What?”, “How much?”, “How well?”, “For how long/how many times?” and “Where?” of the object of study. In our case we suggest giving a higher weight to the area-related FU because we are looking at agricultural activities as multifunctional production systems, not limited to the provision of food. In other words, choosing the area-related FU allows to account for the other ecosystem services provided by the act of cropping within a landscape and a socioecological system.

The comparison with OASIS results supports this perspective. In fact, under the frame of OASIS analysis, it emerges that from the agroecological practices adoption standpoint, organic farms are decidedly more advanced than conventional ones, particularly RO. As already witnessed by the analysis of economic aspects, the adoption of agroecological practices entails a tradeoff between economic and “other” yield (Tittonell et al. 2020).

In fact, a clear tradeoff pattern can be seen in terms of biodiversity (left side of the graph) where the two organic farms outperform the conventional ones, with both the organic and conventional farms obtaining scores near the respective sub-group means. This is once more in contrast with the results of LCA, namely with the results expressed as mass-related FU. The clear distinction of preferability between conventional and organic management systems that occurs when adopting the mass-related FU, cannot be seen in the case of area-related FU. The gap between the mass-related and the area-related FU between organic and conventional is due to the complexity and variability of choices that the farmers face during the rice cultivation season and could be explained in terms of tradeoffs in biodiversity indicators as captured by the OASIS methodology. LCA methods, while accounting for fertilizers and pesticides from a GHG emission standpoint, overlook significant risks to various organisms. These impacts affect vertebrates in rice fields and surrounding habitats, as shown in studies from the Philippines (Pingali e Roger 1995) and South America (Vermeer 2019). Other previous studies report that changes in irrigation

structures further disrupt ecosystems, impacting fauna, that rice field ecosystems host diverse primary producers, including over 1800 weed species, with algae being vital for soil fertility, highlighting the complex ecological dynamics within these agroecosystems (Bambaradeniya e Amarasinghe 2004).

A similar tradeoff pattern can be acknowledged concerning farming resilience. Here the BO, RO, and RC farms show similar scores, higher than BC, and contributing to the best average outcome of the organic versus conventional model. Building on the OASIS indicators contributing to the overall resilience score, these results confirm previous findings on the ability of organic approaches to increase farm resilience (Ariyaratna, Nanayakkara, e Thushara 2023; Panpakdee 2023; Panpakdee, Simaraks, e Sookcharoen 2022; Panpakdee, Limnirankul, e Kramol 2021; Heckelman, Smukler, e Wittman 2018).

On one hand, the fact that the two organic farms obtain the same resilience score as the RC farm, witness the possibility for conventional farming to adopt resilient measures that balance improvements in climatic and economic resilience at the same time. On the other hand, organic farms show the best scores in climatic resilience, adopting a larger set of practices that, as a whole, provide larger contribution to the farming system, i.e. the diversity of their products and income sources, the self-production of seeds, the choice of culturally relevant varieties, the adoption of crop rotations and cover crops.

6.7 Conclusions

In conclusion, the present research provides a snapshot of 4 farms with different management models, and different availability and management of water during a drought year, i.e. 2022, in the Po Valley. We adopted a mixed method approach to analyze several features simultaneously and highlight how different methodologies can explain different shades of sustainability. The results show that the existing tradeoffs in terms of economic and environmental can be explained when the same issue is assessed through a more specialized and holistic method, and that a margin of improvement exists both in organic and conventional schemes. However, the proposed mixed method approach was able to provide a more holistic view and to analyze all the three dimensions of sustainability, helping to understand the tradeoffs and set a goal path for a sustainable management of rice cropping.

In general, we conclude that, with regards to agroecological aspects, environmental impact and biodiversity and resilience, organic farms are more advanced and better projected towards the sustainability objectives of the European Union and the Agenda 2030. This observation is especially important considering that it was obtained in the driest year in the last century and a half. Under these weather conditions, hitherto quite unique but likely to be repeated in the coming years of climatic emergency, the higher adaptive capacities of the organic paddy management system compared to conventional were observed.

From an economic point of view, conventional farms confirm their better performance, even if the specific weather conditions in 2022 limited the added-consumer price for organic farms. The 2022 markets for organic and conventional paddy rice showed very similar selling price due to lack of product. Consequently, the higher production of conventional rice has led to significant margins, not compensated by the usual organic added value.

Overall, the impact of water in rice cultivation remains the most significant issue in environmental and resilience terms. Building on the case study of RO, we may conclude that the highest environmental impact is largely attributable to the additional, and probably not justified, submersion it adopts compared to RC (same availability of water in Rovasenda). Conversely, in Bereguardo, where water was not available, the organic and conventional farms obtain similar impacts. This is a very interesting conclusion, because it paves the way for new studies on water management in relation to the production and fundamental phenological phases of rice.

Limitations of the study are represented by the low number of farms assessed, and by the complexity of comparing systems that differs from a geographical and technical perspective. In order to give a complete interpretation of the comparison between organic and conventional models, further research is needed to increase the observations number, and to increase the coverage of proposed methodologies. For instance, the integration of other methodologies, such as the social LCA, or specific biodiversity assessment methodologies, within OASIS, could provide the possibility to triangulate the results also in these terms.

CHAPTER 7 GENERAL DISCUSSION

As global demand for rice continues to rise, there is a growing need to address the environmental impacts associated with its production. Sustainable rice farming aims to produce this essential crop while reducing ecological strain, conserving resources, and improving economic resilience. Achieving these goals requires not only adjustments in farming practices but also the integration of emerging technologies and scientific advancements. This work explored several key approaches to promoting sustainable rice production: the use of nitrogen-fixing biostimulants, the development of allelopathic rice varieties, the adoption of agroecological assessment systems, and the application of Life Cycle Assessment (LCA) to evaluate environmental impacts comprehensively.

Rice farming traditionally relies heavily on synthetic fertilizers, pesticides, and herbicides, which have profound ecological consequences. Nitrogen fertilizers are essential for high yields but are also notorious for their inefficiency. When applied, a significant portion of nitrogen is lost to the environment through processes like leaching and volatilization, leading to water contamination and greenhouse gas emissions. Nitrogen-fixing biostimulants provide a promising alternative by harnessing the ability of certain microorganisms, such as *Azospirillum* spp., to convert atmospheric nitrogen into forms that plants can utilize. These biostimulants not only reduce the need for synthetic fertilizers but also enhance soil health by promoting microbial biodiversity. By integrating these biological solutions into rice farming, producers can decrease dependency on chemical inputs, lowering costs and reducing environmental impacts.

In addition to nutrient management, sustainable weed control is another critical component of rice production. Conventional rice farming often depends on herbicides, which can contribute to soil and water pollution and lead to selection of herbicide-resistant weed populations. Allelopathic rice varieties offer an eco-friendly alternative, as they naturally produce biochemicals that inhibit weed growth. By selecting and cultivating allelopathic varieties, farmers can reduce their reliance on chemical herbicides, enhancing biodiversity and protecting soil health. This approach aligns with sustainable farming practices by minimizing chemical inputs and allowing for a more balanced ecosystem within the rice fields. Furthermore, research and breeding efforts focused on enhancing allelopathic traits in rice varieties can help tailor these crops to specific environments and weed challenges, making sustainable weed control even more effective.

To understand and improve the sustainability of rice production systems, comprehensive assessment tools are indispensable. The Original Agroecological Survey and Assessment System provides a framework for evaluating various ecological aspects of agricultural operations, such as soil health, water quality, pest dynamics, biodiversity and social aspects. By implementing such assessments, farmers and

researchers can monitor the effectiveness of sustainable practices and make data-driven adjustments. These insights are crucial for refining farming techniques and optimizing resource use. Agroecological assessments thus serve as a foundation for sustainable decision-making, guiding practices that protect and enhance the agricultural landscape.

A broader understanding of environmental impacts across the entire rice production lifecycle is equally vital for sustainability. Life Cycle Assessment (LCA) is a valuable tool for evaluating the cumulative environmental footprint of rice, from field preparation and cultivation to harvesting, processing, and distribution. LCA assesses key metrics such as energy consumption, greenhouse gas emissions, water usage, and waste generation. By highlighting the most resource-intensive and environmentally impactful stages of production, LCA enables farmers, policymakers, and agribusinesses to identify areas where improvements can be made. Integrating LCA into rice production fosters transparency and accountability, promoting the adoption of practices that minimize resource use and reduce emissions. In doing so, LCA contributes to a more sustainable food system, supporting both environmental and societal goals.

In conclusion, sustainable rice production requires a multifaceted approach that incorporates innovative biotechnologies, ecologically based farming practices, and thorough environmental assessments. By leveraging nitrogen-fixing biostimulants, developing allelopathic rice varieties, adopting agroecological assessment systems, and applying LCA, the rice industry can move toward more resource-efficient and environmentally friendly production systems. These advancements not only reduce the ecological footprint of rice farming but also support global efforts to build resilient food systems capable of meeting the needs of a growing population. Through these integrated practices, sustainable rice farming can contribute to a healthier planet, promoting a future where agriculture and the environment are in harmony.

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CHAPTER 9 APPENDIX A

Table 6.1.A Cropping practices: operations and products used in Bereguardo Organic – BO; Table 1.B Cropping practices: operations and products used in Bereguardo Conventional – BC; Table 1.C Cropping practices: operations and products used in Rovasenda Organic – RO; Table 1.D Cropping practices: operations and products used in Rovasenda Conventional – RC.

A – Practices in BO

OPERATION	INPUT	PRODUCT	AMOUNT	UNIT	FUEL	UNIT
Manuring	Manure	Manure	30	Ton/ha	30	l/ha
Plowing			1		70	l/ha
Harrowing			2		20	l/ha
Sowing	Seed	Bald rice seed	200	Kg/ha	10	l/ha
Pruning machine			1		20	l/ha
Harvest			2,5	Ton/ha	50	l/ha

B – Practices in BC

OPERATION	INPUT	PRODUCT	AMOUNT	UNIT	FUEL	UNIT
Manuring	Manure	Manure	30	Ton/ha	30	l/ha
Harrowing			1		25	l/ha
Sowing	Seed	Barone rice seed	180	Kg/ha	10	l/ha
Pre -emergency weed control	Alcance	Clomazone 43 g/l	2,8	L/ha	36	l/ha
		Pendimetalin 298 g/l				l/ha
Fertilization	Npk 23-0-30	Npk 23-0-30	225	Kg/ha	10	l/ha
Post-emergency weed control	Beyond	Imazamox 40 g/l	1,1	L/ha	36	l/ha
	DASH	Metil-oleato e metil-palmitato 37,5 g	1	L/ha		
	MARINS	MCPA 200 g/l	1,5	L/ha		

	PERMIT	Halosulfuron metile 750 g/kg	0,05	Kg/ha		
Fertilization	Npk 23-0-30	Npk 23-0-30	225	Kg/ha	10	l/ha
Fungicide	Amistar	Azoxystrobin puro 250 g/l	1,0	L/ha	36	l/ha
Harvest			4,5	Ton/ha	50	l/ha

C – Practices in RO

OPERATION	INPUT	PRODUCT	AMOUNT	UNIT	FUEL	UNIT
Plowing			1		70	l/ha
Fertilization	Hoof and horn	Nitrogen 14%	250	Kg/ha	10	l/ha
Harrowing			2		25	l/ha
Levelling			1		12	l/ha
Sowing	Seed	Ryegrass seed	50	Kg/ha	10	l/ha
Fertilization	Potassium sulfate	Potassium 50%	180	Kg/ha	10	l/ha
Sowing	Seed	Carnaroli rice seed	180	Kg/ha	10	l/ha
Fungicide	Thiopron	Sulfur	5	L/ha	36	l/ha
Fungicide	Thiopron	Sulfur	5	L/ha	36	l/ha
Fungicide	Thiopron	Sulfur	5	L/ha	36	l/ha
Harvest			2	Ton/ha	50	l/ha

D – Practices in RC

OPERATION	INPUT	PRODUCT	AMOUNT	UNIT	FUEL	UNIT	
Plowing			1		70	l/ha	
Levelling			1		12	l/ha	
Harrowing			1		25	l/ha	
Fertilization	Diammonium phosphate + Hoof and horn	18% Nitrogen	180	Kg/ha	10	l/ha	
		46% Phosphorus					
		14% Nitrogen	330	Kg/ha			
Pre-sowing weed control	Stratos	Cycloxydim	2,2	L/ha	36	l/ha	
Sowing	Seed	Carnaroli rice seed	190	Kg/ha	10	l/ha	
Post-emergency weed control	Aura	Profoxydim	0,3	L/ha	36	l/ha	
	DASH	Metil-oleato e metil-palmitato 37,5 g	0,9	L/ha			
	CLINCHER ONE	Cialofop-buthyl 200 g/l	1,5	L/ha			
	KARATE	Lambda cyhalothrin 9,48 g	0,125	L/ha			
Fungicide	Amistar	Azoxystrobin puro 250 g/l	1,0	L/ha	36	l/ha	
Fungicide	Amistar	Azoxystrobin puro 250 g/l	1,0	L/ha	36	l/ha	
Harvest			4,5	Ton/ha	50	l/ha	

Table 6.2.A Irrigation calendar for the four target farms in 2022: Bereguardo Organic – BO; Table 2.B Irrigation calendar for the four target farms in 2022: Bereguardo Conventional – BC; Table 2.C Irrigation calendar for the four target farms in 2022: Rovasenda Organic – RO; Table 2.D Irrigation calendar for the four target farms in 2022: Rovasenda Conventional – RC.

A - Water management in BO

OPERATION	START	FINISH	SUBMERSION DAY	DRY DAY

Sowing	30/05/2022	30/05/2022		
Submersion 1	20/06/2022	23/06/2022	3	
Dry 1	21/06/2022	09/07/2022		15
Submersion 2	10/07/2022	13/07/2022	3	
Dry 2	14/07/2022	29/07/2022		15
Submersion 3	30/07/2022	02/08/2022	3	
Harvest	22/10/2022	22/10/2022		39
Tot	10/09/2022	10/09/2022	9	69

B - Water management in BC

OPERATION	START	FINISH	SUBMERSION DAY	DRY DAY
Sowing	15/04/2022	15/04/2022		
Submersion 1	12/05/2022	15/05/2022	3	
Dry 1	16/05/2022	03/06/2022		18
Submersion 2	04/06/2022	07/10/2022	3	
Dry 2	07/06/2022	25/06/2022		18
Submersion 3	26/6/2022	02/07/2022	6	
Harvest	10/09/2022	10/09/2022		70
Tot			12	106

RO

OPERATION	START	FINISH	SUBMERSION DAY	DRY DAY
Sowing	28/04/2022	28/04/2022		
Submersion 1	28/04/2022	10/05/2022	12	
Dry 1	11/05/2022	26/05/2022		15

Submersion 2	27/05/2022	29/07/2022	63	
Dry 2	30/07/2022	06/08/2022		7
Submersion 3	07/08/2022	06/09/2022	30	
Harvest	03/11/2022	03/11/2022		58
Tot			105	80

RC

OPERATION	START	FINISH	SUBMERSION DAY	DRY DAY
Sowing	11/04/2022	11/04/2022		
Submersion 1	21/05/2022	28/05/2022	7	
Dry 1	28/05/2022	09/06/2022		12
Submersion 2	10/06/2022	14/10/2022	126	
Dry 2	15/10/2022	21/10/2022		6
Harvest	22/10/2022	22/10/2022		8
Tot			133	26

Table 6.3 – OASIS criteria and indicators used in the analysis.

Criterion	Sub-criterion	Indicator	Sub-indicator
1. Adoption of agroecological practices	Crops	Use of agroecological soil tillage techniques	
		Use of plant reproductive material adapted to low input systems	
		Agroecological soil fertility management	
		Agroecological crop pest management	
		Agroecological crop disease management	
		Agroecological weed management	
		Maximisation of soil cover	
	Natural resource and agroforestry	Efficient water management	
		Favourable microclimate management	
		High level of adoption of agroforestry	
2. Economic aspects	Production cost	Minimised variable costs	Irrigation water
			Synthetic fertilizers
			Organic fertilizers

			Pesticides
			Growth stimulators and other plant products
			Effective microorganisms
			Biological control products
			Seeds
			Employee wages
			Power
			Plastic
			Agricultural machinery
		Minimised fixed costs – investments	Infrastructure
	Revenue	High proportion of quality enhancement product valorisation practices	
		High proportion of locally or self-processed products	
		Short marketing chain	
		Local marketing chain	
		High level of diversification of activities	
	Income	Satisfaction with economic benefits from farming activities	
		Similar or higher benefits compared to other farmers	
3. Sociopolitical aspects	Labour conditions and rights	Job security	
		Stability and wages	
		Inclusion of people at risk of social exclusion	
	Cooperation and networks	Participation in networks and associations	
		Participation in solidarity economy projects (non-monetary)	
		Support and education on agroecology	
		Transparency	
	Local culture and knowledge	Preservation of local culinary traditions	
		Conservation of seeds and local breeds	
	Life quality	Workload	
		Stress level	
		Time for family and social relationships	
		Time to acquire new knowledge	
		Self-consumption	
	Farm viability	Future prospects for the company	
		Inheritance and succession	
4. Impact on environment and biodiversity	Environmental impact	Pollution	
		Soil carbon management	
		Soil erosion	
		Soil salinization	
		Soil compaction	
	Impact on biodiversity	Presence of ecological infrastructures	
		High-nature value farming (HNVf)	
		Level of agrobiodiversity	
5. Resilience	Climatic resilience	Soil carbon budget optimization	
		Maximal use of stress-tolerant species, breeds, and cultivars	
		Favourable microclimate management	
		Maximisation of soil cover	
		Maximisation of agrobiodiversity	
		Efficient water management	

	Economic resilience	High level of diversification of products	
		High level of diversification of activities	
		High level of diversification of clients	
		Short and local food marketing chains	
		Good temporal distribution of revenue	
		Low share of subsidies in gross farm income	
		Ability to attract and keep motivated workforce	
		High level of autonomy from commercial inputs	Use of water
			Plant material
			Commercial nitrogen
			Insecticides
			Herbicides
			Fungicides
			Biological control agents
			Workforce
			Power