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Agro-environmental performance of alternative agronomic practices for improving the sustainability of temperate rice cropping systems

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Abstract

Rice cultivation in temperate agro-ecosystems will face numerous challenges in the coming decades to increase its agro-environmental sustainability. In an effort to increase crop yields and improve production efficiency, conventional agricultural practices often have a major impact on the natural environment. Therefore, there is a need to develop and introduce new strategies that allow rational and optimal use of water and land resources to adapt to climate change and reduce impacts on the environment. In this context, this thesis focused primarily on the adoption of alternate wetting and drying (AWD), a water-saving technique that alternates periods of flooding with periods of drying during the cropping cycle. Attention was also devoted to alternative techniques for paddy soil management particularly conservation tillage (i.e. minimum and no tillage). The aims of this thesis were: (i) to evaluate the impact of AWD on rice agronomic performances, grain quality and greenhouse gas (GHG) emissions; (ii) to assess the influence of water management on the availability of nitrogen (N) for plant uptake from different sources, including mineral N fertilizers, in order to limit losses and optimize N uptake and use efficiency; (iii) to investigate the influences of conservation tillage practices on rice productivity and soil organic carbon (C) stock in the medium-term.

This thesis demonstrated that AWD has the potential to mitigate GHG emissions from paddy fields while maintaining optimal agronomic performance in temperate rice cropping systems. Therefore, AWD may represent a viable alternative to continuous flooding to improve the agro-environmental sustainability of temperate rice cropping systems. The important insights provided regarding the infuence of water, crop

residue and N fertilization management and their interaction on the contribution of different N sources to plant uptake may contribute to identify suitable fertilization practices that favour plant N uptake during the early stages of rice growth under AWD.

Conservation tillage can contribute to increase sustainability of temperate rice systems. Minimum tillage uses production resources more efficiently compared to conventional tillage (i.e. ploughing) and sustains soil fertility by promoting organic matter and N inputs, facilitating soil aggregation and preventing soil compaction. No tillage has some limitations that hinders its adoption, such us yield reductions and excessive soil compaction.

The present thesis demonstrated that higher yields and increased resource-use efficiencies are not necessarily conflicting goals in rice cultivation. This work provides useful insights at field scale, providing a holistic evaluation and leading to the quantification of key agro-ecological indicators which can be of extreme importance for the management of temperate rice cropping systems. Therefore, the understandings and results obtained in this thesis provide practical implications on innovative management of rice paddies.

1. General introduction

Agriculture is currently facing important challenges related to the provision of sufficient and healthy food for a growing population and adapting to climate change (e.g. water scarcity), while also minimizing environmental consequences (i.e. mitigation) (Foley et al., 2011; Linquist et al., 2015; Rockström et al., 2017). The world population is expected to reach 9.6 billion people in 2050 (UN, 2013), and trends in consumption patterns are expected to further increase the demand for food, exerting notable pressure on production systems (Alexandratos and Bruinsma 2012). Estimates suggest that crop production will need to double (2.4% annual increase) by the year 2050 to meet global demand (Ray et al., 2013). An analysis of the current increasing trends in crop yield however show that these needs will not be easily met (Grassini et al., 2013; Bernard et al., 2017). Agricultural intensification, whereby higher yields per unit of land area are obtained, is considered necessary to achieve this goal (Pretty & Bharucha, 2014, Godfray & Garnett, 2014; Tseng et al., 2020). However, intensification may have negative environmental implications such as non-point pollution and increased greenhouse gas (GHG) emissions that could further exacerbate climate change and environment degradation (Matson et al., 1997; Vitousek et al., 1997). This has led to a strong drive to aim for sustainable intensification (Godfray et al., 2011; Garnett et al., 2013) whereby higher yields are achieved without (or with limited) damage to the environment, thereby meeting the dual goals of protecting natural resources while ensuring global food security.

Rice (*Oryza sativa* L.) is the staple food for nearly half of the world's population which makes it an important crop grown on an area of around 165 M ha (Van Nguyen and Ferrero, 2006; FAOSTAT, 2023). Due to the climate, European temperate rice production is concentrated within limited areas across a small number of countries. Rice production in Europe covers about 360.000 ha (FAOSTAT, 2023), almost totally located in few southern European countries, mainly in Italy (60.9% of total area) and Spain (15.6%), and to a lesser extent in Greece (8%), Portugal (7.6%), France (3.3%), Bulgaria (3%), Romania (0.9%) and Hungary (0.7%). Though rice production in the European Union (EU) is comparatively smaller to the total global production (0.4 % of the global rice production), rice farming systems in some of the European regions have a long tradition and have important economic, cultural and landscape relevance at both local and regional scale. In Italy, rice production is mainly concentrated in the north-western Po Valley, covering 218.000 ha (FAOSTAT, 2023). In Vercelli, Pavia and Novara provinces, rice cultivation constitutes about 81% of the land use and has a long tradition of cultivation dating back to the 15th century, and that was developed alongside the establishment of a dense network of historic water channels (Pinto et al., 2002). In this area, rice is generally cultivated in specialized farms in which the fields that are permanently flooded during most part of the growing season (from April-May to August-September), and with a high level of mechanization and chemical inputs, especially herbicides but also nitrogen (N) fertilizers, fungicides and insecticides. Yields range from 6 to 7 t ha^{-1} (Ferrero and Tinarelli, 2008), depending on the type of cultivar (i.e. round, Long A or Long B grain size) that mostly belong to *Japonica* rice varieties. In this area, water management for rice cultivation creates a unique agricultural landscape providing important habitats for many organisms including migratory waterbirds (King et al., 2010), and for this reason flooded paddies are important for biodiversity conservation and artificial wetland maintenance in Europe.

However, the productivity and sustainability of rice and rice-based systems are nowadays threatened by several factors including: (1) the increasing scarcity of natural resources (land, water), (2) the low efficiency of inputs (fertilizer, water, herbicides, etc.) combined with the emerging energy crisis, rising fuel and fertilizer prices, all leading to rising cost of cultivation, (3) the pressing need to reach higher environmental and food safety standards by reducing the environmental impact of rice cropping and maintaining elevated grain quality, and (4) emerging socioeconomic changes such as urbanization, preference of non-agricultural work, concerns about farm-related pollution (Ladha et al., 2009; Prasad et al., 2017). These threats are further confounded by climate change and the need of cropping systems to adapt to more frequent biotic and abiotc stress linked to change (Bocchiola, 2015).

1.1. Rice cultivation and climate change

In an effort to increase crop yields and improve production efficiency, conventional agricultural practices have often resulted in an important impact on the natural environment, both locally and globally. They often contribute to soil, water, and air quality deterioration, a decline in arable land, biodiversity, and ecosystem functioning and stability, and to the emissions of greenhouse gases (GHG) that cause global warming and contribute to climate change (Foley et al., 2011; Linquist et al., 2012b; IPCC, 2022). At the same time, agricultural productivity is influenced by climatic change-related issues that often threat the economic sustainability of the rice sector in numerous ways. These include, for example, changes in plant growth and development as well as biotic stress factors including new pests and diseases, variations in annual rainfall and seasonal rainfall patterns leading to an increase in the frequency of extreme drought and water shortage events, an increasing incidence of temperature anomalies (e.g. heat waves) and increasing soil salinity in coastal regions due to variations in sea levels (Kraehmer et al., 2017; FAO, 2022). Therefore, improving the sustainability of rice production depends, on one hand, on the capability of cropping systems to adapt to climate change, and on the other, on the adoption of agronomic practices that effectively mitigate the contribution of rice cropping to climate change (Fig. 1.1; Hussain et al., 2020). For this reason, climate change has become a challenging focus for researchers, farmers, and policy makers alike.

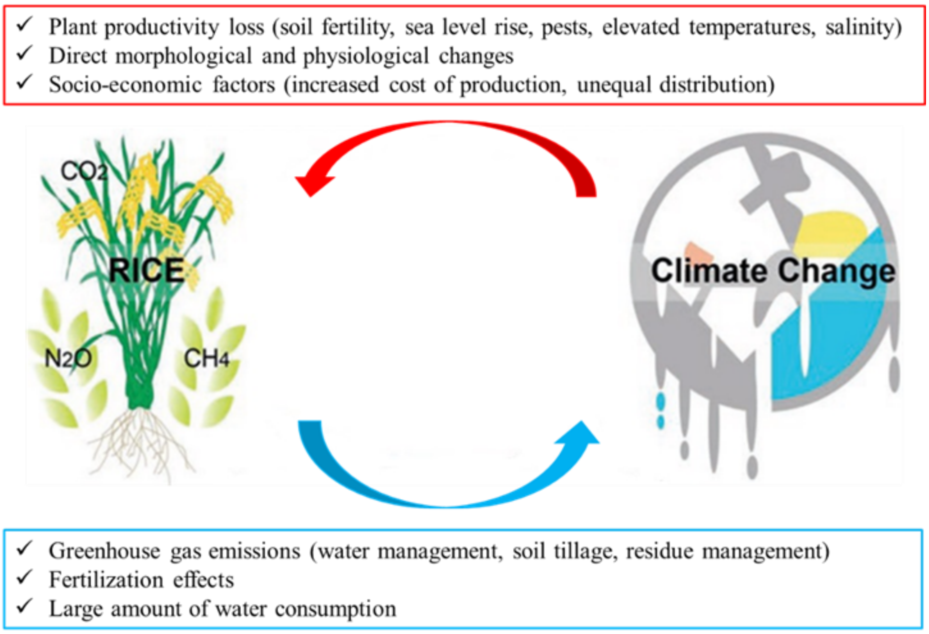

Figure 1.1 Role of rice cultivation on climate change and counter effects of climate change on rice (source: adapted from Hussain et al., 2020).

1.2. Emerging challenges to rice cultivation in Europe

The increasing demand for high crop production within a limited cultivation area is indeed placing a strong pressure on the European rice sector for achieving higher production yields, consequently inducing most farms to adopt intensive rice monoculture systems. However, the pressures resulting from climate change as well as the need to reduce the inputs and environmental impact of rice cropping systems while producing high quality and safe food, is threatening the sustainability of this important sector. Thus, the most important emerging challenges for soil and agricultural management in rice agro-ecosystems, also considering the impact of climate change, include: (1) matching the high demand of irrigation water resources with their decreasing availability; (2) balancing the need to reduce mineral inputs and environmental impacts (e.g. GHG emissions) while sustaining crop yields; (3) enhancing grain quality and safe food production (e.g. reducing metal(loid) grain contents); and (4) protecting soil resources by avoiding the loss of paddy soil fertility (Kraehmer et al., 2017).

1.2.1 Matching demand for water resources with their reduced availability

Water is the primary resource determining the success of the rice crop. About 75% of total rice production comes from irrigated lowlands (Bouman et al., 2007a). The conventional rice irrigation management (i.e. water seeding and continuous flooding until few weeks before harvest) requires copious volumes of water (Mayer et al., 2019). Approximately 2500 L of water are required to produce 1 kg of rice grain (Bouman et al., 2009). Irrigated rice receives an estimated 34–43% of the total world’s irrigation water or about 24–30% of the entire world’s freshwater resources (Surendran et al., 2021). Moreover, rice systems are characterized by a low water use efficiency of around 50% (Bouman and Tuong, 2021).

Water availability, especially in Mediterranean areas, is likely to decline under the projected climate change scenarios. This is mainly due to a decrease in rainfall and snow, together with an increase in the occurance of temperature anomalies that together lead to more frequent and severe water scarcity and drought events (Giorgi and Lionello 2008; Hoerling et al. 2012; Zampieri et al., 2019), resulting in the loss of stable production of rice. Moreover, the competition in water demand between agriculture and non-agricultural sectors (domestic, industrial, and environmental) has become acute (Rosegrant et al., 2009; Hanjra and Qureshi, 2010). At global level, it is expected that by 2050 several million hectares of currently lowland irrigated rice systems will experience water scarcity (Bouman et al., 2007a) leading to important economic losses, and with some current water management strategies becoming no longer viable.

1.2.2. Reducing environmental impacts of rice cropping while sustaining crop yields

Climate change is mostly driven by the influence of rising temperatures in the earth’s atmosphere, with increasing atmospheric concentrations of greenhouse gases being mainly responsible for these changes. Agricultural production accounts for approximately 13% of global anthropogenic emissions (Ranganathan et al., 2016). Methane (CH_4) and nitrous oxide (N_2O) emissions from rice fields have become a major concern over the last century. Estimates of global CH_4 emissions from paddy fields alone range from 31 to 112 Tg year^{-1}, accounting for up to

19% of the total global CH_4 flux (Win et al., 2020). After livestock, rice production is the second largest contributor of agricultural CH_4 emissions. Furthermore, 11% of global agricultural N_2O emissions come from rice fields (Win et al., 2020). Under flooded rice paddy conditions, CH_4 is produced due to anaerobic conditions, while N_2O is produced as a result of different microbial processes including nitrification–denitrification, with applied fertilizer N being the primary contributor both under aerobic upland and anaerobic lowland conditions. The contributions of CH_4 and N_2O to global warming are significantly higher than carbon dioxide (CO_2); over a 100-yr time horizon, CH_4 and N_2O are 28 and 265 times more powerful than CO_2 in forcing temperature increases, respectively (Myhre et al., 2013). Accounting for both CH_4 and N_2O emissions, rice systems also have higher global warming potential (GWP) than other cereals (Linquist et al., 2012a), indicating they emit more GHG per unit of yield. In particular, the production of 1 kg rice returns 0.71 kg CO_2 equivalent (CO_2-eq) emissions to the environment as compared to 0.27 kg CO_2-eq emissions per kg production of other cereals (Kumar et al., 2022).

Until now reductions in GHG emissions by the EU agricultural sector have been limited, despite several measures were taken to incentivize practices with positive impacts on climate change mitigation. However, new EU targets and policy initiatives such as the Green Deal and Farm to Fork strategies, as well as the increasing role of soil carbon (C) sink as mitigation strategy set by the 2015 Paris Agreement and COP26 Glasgow conference, and the increasing perception of consumers of the impact of the agri-food sector on GHG emissions, will drive the agricultural sector towards reinforcing its role in the climate mitigation. GHG emissions from rice fields are substantial and very sensitive to management practices. Therefore, rice could be an important target for mitigating GHG emissions (Wassmann et al., 2004).

1.2.3. Reducing mineral fertilizer inputs and improving their use efficiency

Rice crop uses about 21–25% of the total nitrogen (N) fertilizer consumed globally (Prasad et al., 2017). The great (over)use of mineral fertilizers has resulted in the widespread degradation of natural resources and disturbance of global nutrient cycles (Robertson and Vitousek, 2009; Schlesinger, 2009). Increasing the application of N fertilizers has been the main approach for boosting yields in previous decades, but this has resulted in a low N use efficiency and widespread pollution (Cassman et al., 1998). Based on global estimates, in irrigated rice fertilizer N recovery by the crop averages 46% (Ladha et al., 2005), with more than 50% of applied N not being assimilated by the rice plant. Most of this N is lost through different mechanisms including ammonia volatilization, surface runoff, nitrification–denitrification and leaching.

These biochemical, physical, and microbial processes are influenced by soil water conditions. Therefore, by changing soil water and air equilibrium, water management in paddy fields strongly affects the availability of N for plant uptake and thus the use efficiency of mineral fertilizers. Building on these considerations, it is necessary to develop and adopt cultivation practices to optimize fertilizer-N management in order to increase fertilizer use efficiency and reduce N losses from the system.

1.2.4. Enhancing rice grain quality and food safety

Another important topic related to the production of safe and high-quality rice is the occurrence of potentially toxic elements (PTE) in rice grain. Rice potentially accumulates a much higher concentration of arsenic (As) and cadmium (Cd) in the shoots and grains compared with other cereals (wheat, barley and maize) which makes rice contamination by As and Cd a global environmental health concern (Mandal and Suzuki, 2002; Hu et al., 2013). The accumulation of As and Cd by plants is related to their bioavailability and mobility in the soil, that depend on the soil redox potential and pH amongst other factors. Therefore, water management in paddy fields during rice cropping affects As and Cd bioavailability and their uptake by rice plants (Li et al., 2009; Zhang et al., 2019). Flooding generally limits Cd uptake and content in different parts of rice plants, but markedly increases As bioavailability and, thus, its absorption. On the other hand, water management practices that include soil aeration can increase Cd content in grain while reducing As content (Rinklebe et al., 2016). Therefore, water irrigation system must be carefully managed to reduce the availability of these heavy metals in the soil and produce safer foods.

1.2.5. Protecting soil resources by enhancing paddy soil fertility

The concentrations of GHG in the atmosphere and subsequently global climate change, may be greatly affected by slight changes in soil organic carbon (SOC) stocks (Routh et al., 2014). Anaerobic conditions induced by flooding slow down organic matter decomposition, and thus paddy soils can potentially sequester C (Wu et al., 2011). While rice paddy areas worldwide represent 9% of the global cropland, they accumulate

14% of total SOC pool in croplands (Liu et al., 2021). Soil organic matter (SOM) is an essential eco-system component, the dynamics of which are affected by soil management practices. The fuctions of SOM in improving soil fertility for sustaining plant growth through improving water, nutrient and air availability has been known for a long time. Recently, emphasis has been given to the environmental role of SOM, mainly concerning its function as a sink of atmospheric CO_2 through C sequestration (Valkama et al., 2020). Therefore, the aim of soil management systems should be to maintain or increase SOM stocks. However, due to the reduced organic matter inputs into the soil through inappropriate crop residues management, no crop rotations, low utilization of manure and organic fertilizers (due to absence of livestock farming in the rice area), and increased decomposition rates from the conventional tillage practices (Lemus and Lal, 2005; Hussain et al., 2020), agriculture can result in substantial losses of SOM from arable land reaching up to 20–40% of SOM loss (Zaman et al, 2021). During the last century, intensive and continued tillage practices have caused a worldwide decline in SOC stocks thus increasing CO_2 emissions to the atmosphere (Blanco-Canqui and Lal 2008; Lopez et al. 2012). It is estimated that as much as 60% of SOC in temperate regions and 75% of SOC in the tropics have been depleted by conventional tillage, contributing about 23% of the total GHG concentration in the atmosphere (IPCC 1996; Lal 2004). Conventional tillage can increase GHG emissions from rice fields by affecting soil properties (soil porosity, soil temperature, soil moisture, etc.) and the mechanical breakdown of soil aggregates, causing the release of protected organic C fractions (Hussain et al., 2015). Therefore, despite conventional tillage practices (i.e. ploughing) contribute to achieving high grain yields, their sustainability in rice cropping systems has often been questioned mainly because of their negative effects on SOM mineralization, soil physical, chemical and biological fertility (Lal, 1993; Corsi et al., 2012), which in turn led to low crop yields and low water and fertilizer use efficiency (Khursheed et al., 2019). Therefore, agricultural practices that reduce soil degradation are essential to improve soil quality and agricultural sustainability in rice cultivation.

1.3. Strategies for handling the effects of climate change on rice production

The aforementioned challenges are strongly linked to the need of rice cropping systems to mitigate climate change through the reduction of GHG emissions and the increase in soil C stocks, while at the same time adapting to the effects of climate change.

Several studies showed that rice farms can offer a huge potential for reducing emissions, primarily CH_4 and N_2O, and increasing soil C stocks, thereby supporting the global commitment to reduce GHG and to mitigate climate change in general (OECD, 2021; Lehner and Rosenborg, 2021; Lynch and Garnett, 2021). In fact, the relative mitigation potential for rice (36%) is much higher than that of livestock (9%), and other croplands (3%; Roe et al., 2021).

Therefore, to face the challenges of growing rice under climate change, it is necessary to modify agricultural practices in temperate rice agro-ecosystems and introduce innovative technologies and strategies at farm and field scale to increase the sustainability and resilience of rice farming systems to climate change. Innovation in rice cultivation is mainly related to strategies that allow rational and optimal use of land

and water resources, such as irrigation management and conservation agriculture (CA).

1.3.1. Water management

Water management practices alternative to continuous flooding, the typical irrigation management that involves paddy field flooding from rice seeding to maturity phases, are nowadays highly recommended for concurrently enhancing water use efficiency (WUE), mitigating GHG emissions, improving nitrogen use efficiency (NUE) and safeguarding environmental quality in temperate rice agro-ecosystems (Carrijo et al., 2017). The introduction of water-saving techniques is also necessary to cope with the effects of climate change such as water scarcity and the increasing frequency of drought events. At the same time, these alternative management techniques should not jeopardize rice production and global food security and safety. In the last decades, the adoption of water management practices that involve intermittent irrigation or keeping soil moist but not continuously water saturated (also known as “aerobic” rice) have attracted much attention from farmers, researchers as well as policy makers (Li et al., 2023). Various water-saving irrigation technologies have been developed over the past 20 years to reduce irrigation water and enhance water productivity, but among them, the most widely accepted technique is the alternate wetting and drying (AWD) irrigation practice (Lampayan et al., 2015; Shekhar et al., 2017). AWD is a water-saving technique where instead of maintaining paddy fields continuously flooded, wetting and drying cycles are introduced by allowing the ponded water to recede and drain until a predetermined threshold of soil water potential is reached before re-flooding again. It has been suggested that intermittent irrigation or AWD can reduce water use in rice cultivation by 15-25 % without affecting yields and can lower CH_4 emissions by 30-70 % (Pittelkow et al., 2015; Carrijo et al., 2017; Enriquez et al., 2021; Mishra et al., 2022). Draining continuously flooded rice paddies once or more during the rice-growing season would reduce global emissions by 41 Tg of CH_4 (Yan et al., 2009). Nevertheless, water-saving techniques may increase N_2O emissions (Sanders et al. 2014; Farooq et al., 2022). In fact, alternating aerobic (dry) and anaerobic (wet) soil conditions in the field may strongly influence N transformation processes. Ammonia volatilization and nitrification processes are favoured under AWD compared to conventional water management practices (Tan et al., 2015). Nitrification-denitrification losses of fertilizer-N are six times greater under AWD than continuous flooding (Dong et al., 2012), therefore water saving practices may reduce the NUE of the system (Devkota et al., 2013). Splitting the application of N fertilizers and immobilization/decomposition/mineralization of straw and soil organic matter-derived N are important factors influencing NUE under AWD irrigation (Hameed et al., 2019). However, the contribution of different N sources to plant nutrition under water saving management still needs to be investigated more in detail.

Introducing periods of soil drying during the growing season to aerate the soil is known to substantially decrease As uptake by rice crops, but can also increase Cd phytoavailability (Bakhat et al., 2017). These water-saving techniques are known and have been already tested in Europe and Italy (Lagomarsino et al., 2016; Mazza et al., 2016; Monaco et al., 2021; Martínez-Eixarch et al., 2021), but few data regarding their applicability are available for local European pedoclimatic conditions, and both practical and scientific knowledge is needed. Further studies are needed to check the applicability of these strategies as common agricultural practices in the Mediterranean area. In particular, a holistic evaluation is needed to assess yield response, the trade-off between CH_4 and N_2O emissions, as well as the effects on metal(loid) uptake by the rice plant.

1.3.2. Conservation Agriculture

CA can be a viable alternative to conventional management in order to reduce agronomic, environmental, and economic impact of European temperate rice cultivation (Perego et al., 2019). The adoption of CA is promoted by FAO as a response to sustainable land management, environmental protection and climate change adaptation and mitigation (Pisante et al., 2015). CA is a system of land and farm management that aims to optimize farming productivity and ecosystem services at the field and landscape levels and to prevent soil degradation. It preserves and enhances soil health and biodiversity. The three basic and interlinked principles of CA are: minimum or no mechanical soil disturbance, maintaining a permanent biomass mulch cover on the soil surface, diversification of species in the cropping system (Hobbs et al. 2008). Among CA practices, conservation tillage is an ecological approach to soil surface management and seedbed preparation, and in its many and varied forms like no tillage, minimum tillage, etc., holds promise for the sustainability of agricultural productivity and the environment. Conversion from conventional to conservation tillage, when this is done in line with the principle of CA, may improve soil structure, increase soil organic C, minimize soil erosion risks, conserve soil water, decrease fluctuations in soil temperature and enhance soil quality and its environmental regulatory capacity (Busari et al., 2015). Carbon sequestration potential of conservation agriculture practices is higher than under conventional tillage (Horwath et al., 2018; Corsi et al., 2012), determining environmental and agronomic benefits such as increase of soil fertility and soil biodiversity. Despite all the benefits, the adoption of conservation agriculture practices is still in an initial phase in the Italian rice area (Perego et al., 2019). Therefore, the agronomic response and the environmental and economic feasibility of these techniques need to be evaluated in the Italian rice cropping systems.

1.4. Aims and objectives of the thesis

This work aimed to test the general hypothesis that improving the agro-environmental sustainability of temperate rice cropping systems is possible through the introduction of new cropping strategies. Therefore, this work focuses on testing several techniques for rice cultivation with the aim of reducing environmental impact, facing the effects of climate change, while maintaining high yields to meet the growing food demand. It focuses mainly on water management techniques alternative to continuous flooding (i.e. Alternate Wetting and Drying - AWD) and alternative techniques for paddy soil management, namely conservation tillage (i.e. minimum and no tillage). Although these techniques are already well known and studied, this thesis aims to fill the knowledge gaps that limit their wider application.

In particular, the key questions include:

1. Can AWD help to increase the environmental sustainability of rice cultivation by reducing GHG emissions and thus help to mitigate climate change?
2. Is it possible to face climate change challenges, such as growing rice with less irrigation water through the adoption of AWD without

compromising grain yield and quality? Is there an important varietal effect?

3. How does N fertilization under AWD need to be managed to reduce N losses from the system and increase its use efficiency?
4. What is the role of conservation tillage in climate change mitigation in Italian rice paddies? Does it have a positive effect on C storage in the soil without negatively affecting rice production?

To answer these questions, this thesis addresses the following objectives:

1. To provide a comprehensive evaluation of the adoption of AWD in temperate rice paddies and the impact on rice productivity, grain quality, and CH_4 and N_2O emissions at field scale.
2. To assess the interactions between water, crop and fertilization management and their influence on the contribution of different N sources to plant N uptake and fertilizer N use efficiency in rice.
3. Evaluation of influences of conservation tillage practices on rice productivity, plant N uptake and soil organic C stocks in the medium-term.

1.5. PhD thesis structure

The present thesis consists of a general introduction and three experimental chapters, each one corresponding to a scientific paper published or submitted to an international peer-reviewed journal, which report the main results of the research activities conducted at the Department of Agriculture, Forest, and Food Sciences (DISAFA) - University of Turin under the supervision of Prof. Francesco Vidotto and Prof. Daniel Said-Pullicino, and, during the first year, also by Dr. Chiara Bertora. Part of the research was performed in collaboration with the

Rice Research Center of Ente Nazionale Risi. The research was funded by the projects RISWAGEST (Innovative water management in paddy fields) and RISTEC (New cultivation techniques for the future of rice cultivation), both financed by the Agricultural and Forestry Research funding programme (2014–2020) of the Lombardy Region.
The full citations of the scientific papers on which the following three chapters are based are as follows:

- **Chapter 2:** Vitali, A., Moretti, B., Bertora, C., Miniotti, E.F., Tenni, D., Romani, M., Facchi, A., Martin, M., Fogliatto, S., Vidotto, F., Celi, L., Said-Pullicino, D., 2024. The environmental and agronomic benefits and trade-offs linked with the adoption alternate wetting and drying in temperate rice paddies. Field Crops Research, 317: 109550. https://doi.org/10.1016/j.fcr.2024.109550.
- **Chapter 3:** Vitali, A., Russo, F., Moretti, B., Romani, M., Vidotto, F., Fogliatto, S., Celi, L., Said-Pullicino, D., 2024. Interaction between water, crop residue and fertilization management on the source-differentiated nitrogen uptake by rice. Biology and Fertility of Soils, 60: 757-772. https://doi.org/10.1007/s00374-024-01794-0.
- **Chapter 4:** Vitali, A., Moretti, B., Lerda, C., Said-Pullicino, D., Celi, L., Romani, M., Fogliatto, S., Vidotto, F., 2024. Conservation tillage in temperate rice cropping systems: Crop production and soil fertility. Field Crops Research 308: 109276. https://doi.org/10.1016/j.fcr.2024.109276.

The effects of AWD on the agronomic and environmental sustainability of water seeded rice cropping systems were evaluated in a field experiment in which AWD was compared to conventional continuous flooding water management (Chapter 2). The hypothesis tested was that **with the adoption of appropriate AWD management, grain yield and quality could be sustained while mitigating the environmental impact of temperate rice systems**. The effects of different water management practices on yield, yield components, plant N uptake, apparent N recovery (ANR), rice grain quality (metal(loid) content in grain), emissions of CH_4 and N_2O and Global Warming Potential (GWP) were evaluated over two years at field scale.

The effects of AWD on the N cycle in paddy soils were further investigated in a mesocosm experiment (Chapter 3), in order to better understand how the interaction between water, crop residue and N fertilization management influence the contribution of different N sources to plant nutrition. Here we hypothesized that **microbial processes driving the source-differentiated N supply for rice uptake during the early growth stages will depend on the interaction between water management, the timing of straw incorporation with respect to flooding and the temporal distribution of mineral N application.** This study aimed at identifying suitable fertilization practices that favour plant N uptake and minimize N losses particularly during the early stages of rice growth under AWD, when most N losses in the field occur. Rice was grown for 60 days in a mesocosm experiment involving a factorial design with (i) two water regimes (continuous flooding vs. AWD) and (ii) three straw and fertilizer managements, during which soil N, porewater chemistry, plant growth and N uptake were evaluated. Source partitioning of plant N between fertilizer-, straw- and soil-derived N was achieved by means of a dual-stable isotope ^{15}N tracing approach.

A third study (Chapter 4) focused on the adoption of conservation agriculture for rice cultivation in continuous monoculture temperate rice systems, and in particular on the comparison among different conservation tillage techniques (minimum and no tillage) and conventional tillage in the medium term. By means of a field experiment, the overarching hypothesis that **conservation tillage can provide high grain yields by increasing soil fertility compared to conventional tillage** was tested. A six-years monocrop rice experiment (2014–2019) was carried out in North-West Italy, comparing three tillage methods including conventional tillage (ploughing – CT), minimum tillage (MT), and no tillage (NT) combined with three N fertilization rates (0, 120 and 160 kg N ha^{-1} $year^{-1}$). The study evaluated yield, yield components, plant N uptake, apparent N recovery (ANR), soil bulk density, total soil organic carbon (SOC) stocks and C and N distribution between different soil organic matter (SOM) fractions.

The findings of Chapters 2, 3 and 4 were then discussed on a broader scale in the conclusions (Chapter 5), which summarize the advantages and disadvantages of alternative strategies for improving temperate rice cropping system agro-environmental sustainability, and highlights knowledge gaps to set the basis for future research.

2. The environmental and agronomic benefits and trade-offs linked with the adoption alternate wetting and drying in temperate rice paddies

2.1. Introduction

Rice is the second most cropped cereal in the world with a production of 776 million tons and a harvested area of 165 Mha in 2022, and is a staple food for more than half of the world's population (FAOSTAT, 2023; Van Nguyen and Ferrero, 2006). Rice cultivation receives 34–43 % of total world water irrigation (Bouman et al., 2007) and is a significant source of greenhouse gas (GHG) emissions (Linquist et al., 2012), due to permanent flooding conditions generally adopted during the cropping cycle. Globally, methane (CH_4) emissions from rice cultivation contribute around 10 % (0.5 Gt CO_2-eq) of the total non-CO_2 emissions from agriculture (5.3 Gt CO_2-eq; FAO, 2020). Field flooding may also affect food safety through metal(loid) accumulation in rice grains, resulting in potential health risks associated with ingestion of arsenic (As) and cadmium (Cd) contaminated rice, especially in countries in which rice is a staple food (Banerjee et al., 2013; Zhu et al., 2008).

Alternate wetting and drying (AWD), which generally involves the frequent alternation between field flooding and drainage during the growing season, has been proposed to improve the agro-environmental sustainability of rice cultivation (Lampayan et al., 2015). AWD adoption may mitigate the negative impacts of continuously flooded rice systems, by reducing water use by 23–33 % (Carrijo et al., 2017) and mitigating GHG emissions, in particular CH_4 by 48–93 % (2015; Martínez-Eixarch et al., 2021). On the other hand, the frequent alternations in redox conditions associated with AWD are known to favour both microbial nitrification and denitrification, increasing nitrous oxide (N_2O) emissions, which has a radiative forcing much higher than CH_4 (Lagomarsino et al., 2016; Verhoeven et al., 2018). Consequently, the overall effect of AWD can be an increase (Lagomarsino et al., 2016) or a decrease in the global warming potential (GWP), as a function of factors that affect CH_4 and N_2O emissions (Mazza et al., 2016; Peyron et al., 2016).

Nonetheless, the benefits and trade-offs associated with the adoption of AWD are expected to be related to the severity and frequency of the drainage events and, in particular, to the threshold moisture level or water table depth reached when the fields are reflooded. Various studies have reported the influence of AWD on rice grain yields as a function of AWD severity and timing during the cropping season, and interactions with rice variety (Carrijo et al., 2017). Generally no significant reduction in grain yields are observed when a safe/mild AWD is applied (i.e., field reflooding is applied when a soil water potential of >–20 kPa or a water level of no more than –15 cm below the soil surface is reached), whereas with more severe AWD thresholds (i.e. soil water potential <–20 kPa), yield gaps as high as 22.6 % have been reported with respect to conventional water management (Carrijo et al., 2017). Some studies have also shown that AWD can increase grain yields compared to continuous flooding (Yang et al., 2009, 2017; Zhang et al., 2009).

Alternation between oxic and anoxic soil conditions under AWD affects the nitrogen (N) cycle with important implications on plant N uptake and N use efficiency of rice plants (Xu et al., 2019). Changes in soil hydrology and redox status with the adoption of AWD could lead to increased nitrification, greater N losses through denitrification, volatilization and leaching, and consequently reduced plant N availability and uptake (Hussain et al., 2015; Pandey et al., 2014; Shekhar et al., 2021). Depending of the severity, AWD was shown to reduce by about 6–12 % (Shekhar et al., 2022), maintain (Cheng et al., 2022; Ku et al., 2017) or improve N use efficiency with respect to continuous flooding (Liu et al., 2013; Ye et al., 2013), probably due to the confounding effects of AWD on the synchronization between water management and fertilizer distribution, and plant root development (Santiago-Arenas et al., 2019; Wang et al., 2016).

Adoption of AWD has also been reported to reduce As availability, plant uptake and its concentration in rice grains, primarily due to the limited mobilization and uptake of As under oxic soil conditions (LaHue et al., 2016; Linquist et al., 2015; Norton et al., 2017a). On the other hand, a general increase in soil redox potentials and an associated decrease in soil pH with field drainage under AWD (Das et al., 2016) may favour Cd accumulation in rice grain (Carrijo et al., 2022; Cattani et al., 2008). The impact of water management strategies on the mobility of other potentially toxic elements, such as nickel (Ni), is still less understood and deserves specific attention. While a decrease in redox potential was shown to enhance Ni release from soil to solution (Rinklebe and Shaheen, 2017), some reports suggest that rice grown in more oxidative soil conditions can accumulate greater Ni concentrations (Norton et al., 2017b). Even here, the severity of AWD cycles is expected to influence metal(loid) availability and uptake (Carrijo et al., 2018), but there is also an important varietal effect linked to the rice genotypes cultivated (Monaco et al., 2021). In order to ensure food safety, the European Union regulates the maximum limits for inorganic As species (iAs) (i.e. 0.15 mg iAs kg^{-1} of white rice; Commission Regulation EU, 2023) and total Cd (0.15 mg kg^{-1}; Commission Regulation EU, 2021) in rice grain, and is currently considering amending the maximum limit of total Ni in husked rice (2.0 mg kg^{-1}). Thus, understanding the influence of water management on grain metal(loid) contents is important for both food safety and to protect the economic sustainability of rice cropping systems and livelihood of farmers.

Most of the studies evaluating the effects of AWD on grain yield and quality as well as on the environmental sustainability of rice paddies focus on tropical and subtropical rice cropping areas (Bouman and Tuong, 2001; Lampayan et al., 2015; Yang et al., 2017), while only a few have investigated the adoption of AWD in European temperate rice cropping systems (Gharsallah et al., 2023; Lagomarsino et al., 2016; Martínez-Eixarch et al., 2021; Mazza et al., 2016; Monaco et al., 2021; Oliver et al., 2019; Orasen et al., 2019; Peyron et al., 2016). Furthermore, to date the extent of AWD adoption in temperate rice systems is still limited and mostly constrained to marginal cropping areas where water availability is already scarce. The diffusion of AWD has been mainly limited by an incomplete appreciation of the linked environmental and agronomic benefits and trade-offs, especially when compared to the more conventional water management practices, as well as due to the paucity of information on the pedoclimatic and hydrological suitability of different rice farming areas to AWD (Sander et al., 2017). Although various studies have evaluated the effects of AWD on rice yields, N dynamics and environmental impacts separately (Monaco et al., 2021; Peyron et al., 2016; Verhoeven et al., 2018), few studies have quantified different agro-ecological indicators simultaneously in order to provide a holistic evaluation of AWD adoption in temperate rice paddies. Furthermore, considering that the severity of AWD adoption in the field and the suitability of paddy soils for AWD management may be rather variable (Nelson et al., 2015), results on the agro-ecological implications of AWD adoption are often contrasting. Most of the studies in temperate regions tested “safe” or “mild” AWD with a low level of severity, and some of them limited the application of AWD cycles exclusively to the vegetative stages to avoid yield losses related to sterility during rice flowering. In addition, all of these experiments, with the exception of Gharsallah et al. (2023) and Martínez-Eixarch et al. (2021), applied AWD in combination with dry seeding and delayed flooding at the tillering stage rather than with water seeding. Recently, Gilardi et al. (2023) have highlighted the benefits of applying AWD in combination with water seeding in Italian rice context. They show how anticipating water use in April-May, when water resources are usually more abundant, may ensure sufficient groundwater recharge in spring thereby reducing the paddy water requirements in June-July when irrigation needs for other crops like corn increase. Since AWD results are influenced by site-specific conditions, there is a need to test AWD with different forms of severity in different regions to enable larger adoption of this technique, and to adapt AWD regimes to local production environments and field scales (Carrijo et al., 2017; LaHue et al., 2016).

Building upon these considerations, this work aims to simultaneously evaluate the agronomic and environmental sustainability of water seeded rice cropping systems under AWD as a function of different severity levels. We hypothesized that:

(1) **AWD, even when applied in a severe way, does not lead to water stress that can compromise grain yield with respect to continuously flooded systems, although some varieties are better adapted than others**;

(2) **the higher N losses that may occur with AWD compared to continuous flooding do not negatively affect N uptake and apparent N recovery**;

(3) **AWD maintains a high quality of rice grain by limiting the availability and plant uptake of metal(loid)s present in the soil**;

(4) **despite the possible increase in N_2O emissions with repeated alternations in redox conditions under AWD, this management mitigates CH_4 emissions and reduces the overall GWP**.

We tested these hypotheses at field-scale over two cropping seasons by comparing two AWD managements, characterized by different severity, with conventional continuous flooding and evaluating yields and yield-related traits, N uptake, grain metal(loid) contents as well as variations in CH_4 and N_2O emissions and their specific contribution to the GWP.

2.2. Materials and methods

2.2.1. Experimental site description

This study was conducted in 2021 and 2022 in the experimental fields of the Rice Research Centre (Ente Nazionale Risi) in Castello d'Agogna (45°14'48''N, 8°41'52''E, NW Italy). The site is located in the western area of the plain of the river Po within the most extensive Italian rice district. The soil of the experimental field was a Fluvaquentic Epiaquept coarse silty, mixed, mesic (Soil Survey Staff, 2014). The topsoil (0–30 cm) was characterized by a loam texture, with a pH in water of 5.6, 11.3 g kg^{-1} organic carbon (C), 1.1 g kg^{-1} total N, 19.5 mg kg^{-1} Olsen phosphorus (P), and cation exchange capacity of 9.6 $cmol_{(+)}$ kg^{-1}. The concentrations of aqua-regia extractable As, Cd and Ni were 13.0, 0.2 and 31.3 mg kg^{-1}, respectively.

The climate is temperate subcontinental, characterized by hot summers and two main rainy periods in spring (April–May) and autumn (September–November). The mean annual temperature was 13.4 °C and 14.7 °C in 2021 and 2022, respectively, higher than the mean over the last 20 years (12.9 °C); during the growing season (May–September) the mean temperature was 21.8 °C and 23.5 °C, respectively (Fig. 2.1). The annual cumulative precipitation over the experimental period was 468 and 357 mm in 2021 and 2022, respectively (Fig. 2.1), lower than the mean over the last 20 years (659 mm).

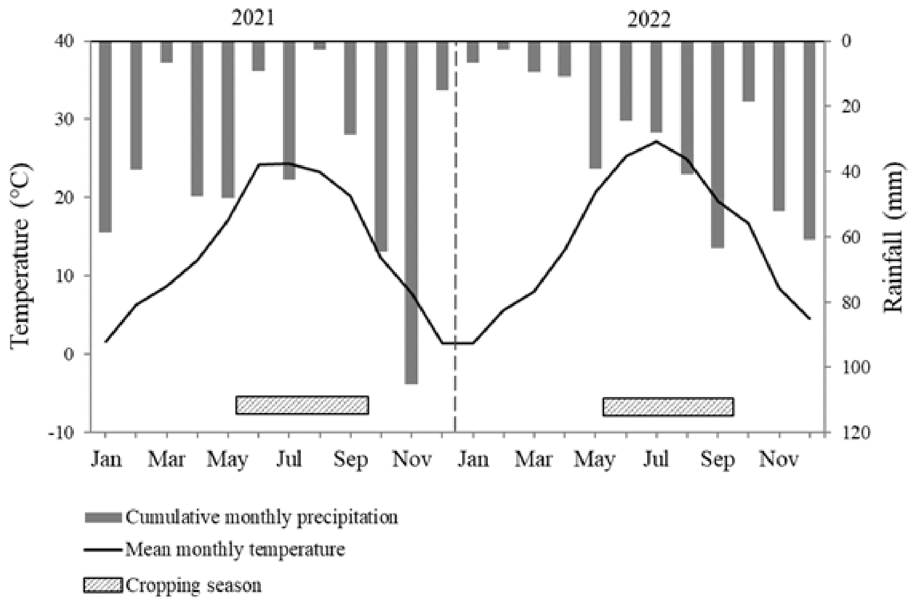

Figure 2.1. Average monthly temperature and total precipitation over the 2021–2022 experimental period.

2.2.2. Experimental design and treatments

The experiment was laid out in a split-split-plot design. The main experimental factor was water management, and included (i) water seeding and continuous flooding (WFL); (ii) water seeding and moderate

AWD (AWD_{safe}; water potential threshold of –5 kPa at 5 cm above ground level); and (iii) water seeding and severe AWD (AWD_{strong}; water potential threshold of –20 kPa at 5 cm above ground level), with two replicate 1500 m^2 plots for each water treatment. In order to manage distinct water regimes in an economically and logistically feasible way, replicate plots for each water management were kept adjacent as described by de Vries et al. (2010) and Miniotti et al. (2016). Packed levees (50 cm above soil surface), covered with plastic film inserted below the soil surface to minimize the lateral movement of water, and two-side canals (25 cm deep) were created to maintain each plot hydraulically independent and to allow the independent management of water level. All plots were maintained with the same water regime during both years of the study.

Every main plot was divided in three 500 m^2 subplots where three varieties were sown, representing the second experimental factor. These included Selenio, Cammeo and CL26, which according to the CODEX classification (FAO and WHO, 2019) based on the grain length, belong to the short, medium and long rice grain groups, respectively. The varieties were selected on the basis of their representativeness in each group and different morphological characteristics. In each subplot, 32 m^2 sub-sub plots were established in which three different N fertilization doses were applied, each replicated twice: (1) *N+* fertilization with a conventional N rate for the different varieties considered (140 kg N ha^{-1} for Selenio and Cammeo, and 160 kg N ha^{-1} for CL26), (2) *N* fertilization with a rate of 40 kg ha^{-1} less than *N+* (100 kg N ha^{-1} for Selenio and Cammeo, and 120 kg N ha^{-1} for CL26), and (3) *N0* fertilization as a non-fertilized control. The N fertilizer (urea, 46% N) was split in 40% of total N applied in pre-seeding and incorporated into the soil by harrowing during seedbed preparation, 30% at tillering stage and 30% at panicle differentiation stage. The timing of N fertilizer application reflected the different development of the crop under the different water managements: panicle differentiation stage in the AWD treatments was delayed by a few days compared to WFL, and consequently the second topdressing N fertilization was also delayed (Table 1). In addition, 42 kg P_2O_5 ha^{-1} (18.3 kg P ha^{-1}) and 114 kg K_2O ha^{-1} (94.6 kg K ha^{-1}) were applied at tillering stage across all treatments.

Soil tillage involved ploughing and laser levelling in the spring and harrowing with a power harrow for seedbed preparation. In all plots the rice crop was established by broadcast water seeding on May 7 and 12 in 2021 and 2022, respectively (Table 2.1), with the same seeding rate (150 kg ha^{-1}) for the three varieties. During winter all plots were maintained drained and fallow following typical practices in the region.

Table 2.1. Crop management under the different water management practices during the two years of the study (2021 and 2022).

Management practice	WFL		AWD_{safe}		AWD_{strong}	
	2021	2022	2021	2022	2021	2022
Spring tillage	15-Mar	14-Mar	15-Mar	14-Mar	15-Mar	14-Mar
Basal fertilization	5-May	10-May	5-May	10-May	5-May	10-May
Field flooding	6-May	11-May	6-May	11-May	6-May	11-May
Seeding	7-May	12-May	7-May	12-May	7-May	12-May
Post-emergence herbicide Treatments (2 application)	8-Jun	20-May	8-Jun	20-May	8-Jun	20-May
	16-Jun	13-Jun	16-Jun	13-Jun	16-Jun	13-Jun
First topdressing N fertilization	17-Jun	14-Jun	17-Jun	14-Jun	17-Jun	14-Jun
Second topdressing N fertilization	7-Jul	4-Jul	12-Jul	11-Jul	12-Jul	11-Jul
Field drainage before harvest	2-Sep	29-Aug	2-Sep	29-Aug	2-Sep	29-Aug
Harvest						
Selenio	29-Sep	20-Sep	29-Sep	20-Sep	29-Sep	20-Sep
Cammeo	24-Sep	19-Sep	24-Sep	19-Sep	24-Sep	19-Sep
CL26	23-Sep	16-Sep	23-Sep	16-Sep	23-Sep	16-Sep

After initial flooding and water seeding, pinpoint flooding method was applied in the WFL treatment (Hardke and Scott, 2013). This involved repeatedly draining and flooding the soil during the seedling stage to promote root extension, avoid soil hardening and keep algal growth under control. After this period, continuous flooding (10 cm of ponding water) was maintained throughout the cropping season, except for two 3-5 d drainage periods at the start of tillering (middle of June) and panicle initiation stage (early/mid July) for fertilizer and herbicide application. In both drainage periods, field flooding was restored within one day from top-dressing fertilization, to avoid significant N losses by ammonia volatilization (Fig. 2.2).

In the AWD treatments, water management was the same as WFL until tillering, and then AWD cycles were applied. Plots were irrigated to a ponding water depth of 10 cm above the soil surface and then the water was progressively left to dissipate through evapotranspiration and percolation until the AWD threshold was reached, after which the plots were reflooded and a new AWD cycle repeated. The hydrological conditions of AWD plots were monitored by measuring (i) soil water potential with four tensiometers (one for each AWD plot) placed at 5 cm depth, (ii) soil volumetric water content with four soil moisture probes (Drill & Drop, Sentek Sensor Technologies, Stepney, Australia) to a depth of 5 cm, and (iii) water table depth with eight piezometers (two for each AWD plot) consisting of perforated PVC tubes of 50 cm length and 15 cm diameter, inserted vertically to a depth of 30 cm from the soil surface. The AWD thresholds adopted were based on previous studies involving *safe/mild* AWD and *severe/strong* AWD (Bouman et al., 2007; Lampayan et al., 2015; Carrijo et al., 2017). The threshold for AWD_{safe} was set at a soil water potential of –5 kPa at 5 cm depth, corresponding to soil volumetric moisture of 40% and a depth of water table of –10/–15 cm, while in AWD_{strong} the threshold was set at a lower soil water potential (–20 kPa at 5 cm depth), corresponding to soil volumetric moisture of 36% and a depth of water table of –20/–25 cm.

In the AWD_{safe} and AWD_{strong} treatments, 6 and 5 flood irrigation events occurred in 2021 while 6 and 7 in 2022, respectively (Fig. 2.2). In 2022, reduced rainfall in the first half of the season and high mean temperatures during the growing season (Fig. 2.1) led to drought and reduced water availability. As a result, slightly more severe AWD thresholds were reached in the second experimental year than in 2021. Net irrigation (mean 2021-2022) applied was 1351 mm in WFL, 1006 mm in AWD_{safe} and 932 mm in AWD_{strong}.

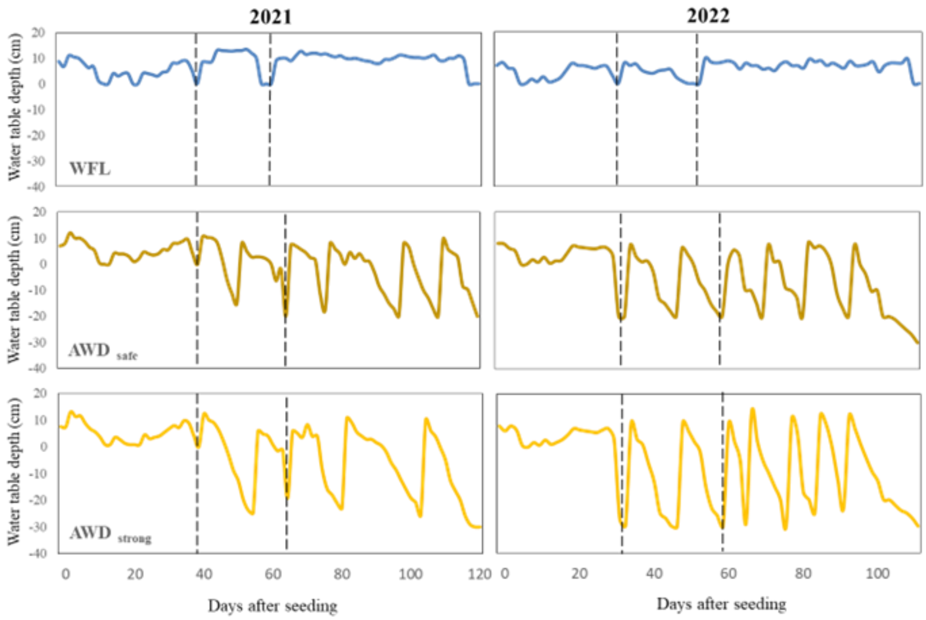

Figure 2.2. Water regime of experimental plots under WFL (water seeding and continuous flooding), AWD_{safe} (water seeding and moderate AWD) and AWD_{strong} (water seeding and severe AWD) in the two years of the study (2021 and 2022). Dashed lines represent the date of topdressing N fertilizations.

Herbicide and fungicide treatments were conducted following the standard practices of the area and were the same for all varieties and water management. When rice reached the ripening stage around 20 d before harvest, all plots were drained and harvest was carried out when the grain moisture was around 20-22% during the last 15 d of September depending on the variety and year.

2.2.3. Sampling and measurements

2.2.3.1. Yields and yield components

Grain yields for all varieties were determined with a combine harvester in each 32 m^2 sub-sub plot. Collected grain was dried, weighed and the values expressed on the basis of a 14% moisture content. Panicle density per m^2 was determined at heading by counting panicle number in three sampling areas (0.25 m^2) for each sub-sub plot. The other yield components (i.e. number of spikelets per panicle, 1000-grain weight and percentage panicle sterility) were measured from 20 panicles randomly sampled in each sub-sub plot. Plant height was measured on the highest tiller of 4 randomly selected plants at the late ripening stage (87 BBCH code).

2.2.3.2. N contents and Apparent N Recovery

Total N content in dried grain and straw samples was determined by elemental analysis (UNICUBE Elemental Analyzer, Elementar, Germany). Total N uptake was obtained by multiplying grain and straw dry weight by their respective N content. Apparent N recovery (ANR) was calculated for *N* and *N+* treatments, according to the following equation by Zavattaro et al. (2012):

$$ANR = \frac{(N\ uptake_N) - (N\ uptake_0)}{F_N} \times 100\%$$

where $N\ uptake_N$ is total plant (grain + straw) N uptake expressed as kg N ha^{-1} for *N* and *N+* rate fertilization, $N\ uptake_0$ is total plant uptake expressed as kg N ha^{-1} in the *N0* treatment, F_N is the amount nitrogen applied with mineral fertilizer (as kg N ha^{-1}).

2.2.3.3. Arsenic, cadmium and nickel contents in grain

Grain metalloid and metal contents (total As, Cd and Ni) were determined on milled white rice grains from plots with standard *N+* fertilization only. Aliquots of milled white rice (0.5 g) were digested with 6 mL 65% nitric acid (HNO_3) and 1 mL 30% hydrogen peroxide (H_2O_2) in a heating block system in 50 mL polypropylene tubes at 95 °C for 2 h. The digested solutions were filtered with 0.45 μm teflon filters after appropriate dilution with ultra-pure water. Total As, Cd and Ni concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS NexION 350X, Perkin Elmer, USA). NIST 1568a and NIST 1568b rice flour were used as certified reference material to ensure the accuracy of analytical procedures for total As and Cd, respectively. Total As and Cd were quantified in the rice grain produced in both years while Ni was only quantified in 2022.

2.2.3.4. Greenhouse gas emissions

CH_4 and N_2O fluxes were measured during the entire growing period in both years for the Selenio variety with standard *N+* fertilization by adopting a non-steady-state closed chamber technique and following the protocol described by Bertora et al. (2018a), with four replicates for each water management (two in each main plot). Stainless steel anchors

(75 × 36 × 40 cm high) were inserted into the soil up to a depth of 40 cm from the soil surface. Chambers were positioned at least 1 m inside the plots and wooden boards were adopted to access the anchors during sampling to avoid soil compaction or crop disturbance. During each flux measurement event, a rectangular stainless steel chamber (75 × 36 × 20 cm high) was sealed over each anchor by means of a water-filled channel, including the growing rice plants within when present. Chambers were covered with a 5 cm thick light-reflective insulation to limit temperature variations inside the chamber during flux measurements, and were equipped with a pressure vent valve designed according to Hutchinson and Mosier (1981), a battery-operated fan to ensure sufficient mixing of headspace air, and a gas sampling port. Steel chamber extensions (15 cm high) were added, when necessary, between anchor and chamber in order to accommodate the growing rice plant throughout the entire cropping season (maximum of four around harvest). Headspace gas samples from inside the chambers were collected by propylene syringes at 0, 10, 20 and 30 min after the chamber closure, and subsequently injected into 12-mL pre-evacuated vials closed with butyl rubber septa (Exetainer® vial from Labco Limited, UK). All gas-sampling events occurred between 10:00-13:00 hrs to minimize variability due to diurnal variations in gaseous fluxes, as also applied by Pittelkow et al. (2013). Collected samples were analyzed for CH_4 and N_2O by gas chromatography on a fully automated gas chromatograph (Agilent 7890A with a Gerstel Maestro MPS2 auto sampler, Santa Clara CA, USA). Gas flux measurements were conducted at weekly intervals with higher sampling frequency in correspondence with fertilization, irrigation, flooding and drainage, when higher fluxes were expected.

Fluxes were calculated from the linear or non-linear (Hutchinson and Mosier, 1981) increase in gas concentration within the chamber headspace with time, as suggested by Livingston and Hutchinson (1995). Cumulative CH_4 and N_2O emissions were determined by linear interpolation of gas emissions across sampling days, assuming a linear trend of emissions in the days between each sampling. Emission factors (EF) for CH_4 for each water management, expressed as kg CH_4 ha^{-1} d^{-1}, were calculated by dividing the cumulative CH_4 emissions over the rice cropping period by the duration of the crop cycle (145 and 131 days in 2021 and 2022, respectively). The overall GWP, expressed in CO_2-equivalent units, was calculated considering a radiative forcing potential relative to CO_2 over a 100-yr time horizon of 28 for CH_4 and 265 for N_2O (Myhre et al., 2013). From the ratio of grain yield (Mg ha^{-1}) and GWP (kg CO_2-eq ha^{-1}), the GHG Eco-Efficiency (kg grain kg^{-1} CO_2-eq) that represents the amount of rice grain obtained per unit GHG emitted, was calculated. Moreover, to better understand the drivers and dynamics of CH_4 emission, soil redox potentials in each treatment were monitored potentiometrically at a soil depth of 10 cm throughout the cropping seasons.

2.2.4. Statistical analyses

All data were tested for normal distribution and homogeneity of variances using the Shapiro–Wilk test and the Levene test, respectively. Data that did not pass the test were log transformed. The Analysis of variance (ANOVA) was performed using the "*lme*" R function to assess significance of water management, variety, fertilization and year and their interactions. When significant ($p < 0.05$), treatment averages were separated through Bonferroni post hoc test. Statistical analysis was performed using R software, version 4.3.0.

2.3. Results

2.3.1. Yields and yield components

No significant effects of *N* and *N+* fertilization were recorded for yield and yield components, and these data were therefore presented as the average between the two fertilization treatments. Water management significantly affected grain yields with unexpectedly lower yields in WFL compared to both AWD managements that showed similar yields, with no differences between the two experimental years (Table 2.2). Significant interaction between water management × variety evidenced a different response of the three tested varieties to water management. In fact, similar grain yields under all water management practices were observed for Selenio and CL26, while higher yields were noted for Cammeo under both AWD managements compared to WFL. AWD_{strong} caused higher straw and total biomass than AWD_{safe} and WFL, although these differences were not consistent over the two years. Water management also significantly affected plant height, with values decreasing in the order $WFL<AWD_{strong}<AWD_{safe}$ in 2021, while in 2022 no significant differences among treatments were observed.

Water management also affected yield components to some extent (Table 2.3). The effects of water management on panicle density showed a significant interaction with year, as in 2021 AWD_{safe} and AWD_{strong} showed higher densities than in WFL, while in 2022 the opposite was true. AWD_{strong} showed higher spikelets per panicle than WFL, while intermediate values were obtained for AWD_{safe}. AWD_{safe} and AWD_{strong}

significantly decreased the 1000 grain weight compared to WFL in 2021 but not in 2022. In general, both AWD managements resulted in significantly higher sterility than WFL, but the effects varied between the three varieties. Although sterility in Cammeo was not affected by water management, Selenio and CL26 showed a higher sterility under AWD_{strong} with respect to WFL, with AWD_{safe} showing intermediate effects in the latter and similar values to AWD_{strong} in the former.

Table 2.2. Performance of the three water managements alone and in interaction with the two years and with the three varieties in terms of grain yield, straw and total biomass, and plant height. Data are presented as average between N and N+ fertilization. Within each parameter, means followed by different letters denote differences among water managements within each year or variety (p(F)<0.05), while the absence of letters suggests no significant differences.

Year (Y)	Variety (V)	Water manag.[a] (WM)	Grain yield (Mg ha^{-1})		Straw yield (Mg ha^{-1})		Total biomass (Mg ha^{-1})		Plant height (cm)	
2021		WFL	10.1 ± 0.8		9.0 ± 0.7	c	19.1 ± 1.4	b	71.5 ± 4.8	a
		AWD_{safe}	10.4 ± 0.8		9.7 ± 0.8	b	20.1 ± 1.4	a	68.3 ± 4.7	b
		AWD_{strong}	10.4 ± 0.8		10.4 ± 0.9	a	20.8 ± 1.6	a	70.1 ± 5.4	ab
								a		
2022		WFL	10.4 ± 0.8		10.2 ± 0.8	a	20.6 ± 1.4		68.6 ± 4.4	a
		AWD_{safe}	10.4 ± 0.9		10.0 ± 0.9	a	20.4 ± 1.7	a	67.7 ± 3.8	a
		AWD_{strong}	10.8 ± 1.1		10.4 ± 0.8	a	21.2 ± 1.7	a	69.4 ± 4.0	a
Average		WFL	10.3 ± 0.8	b	9.6 ± 1.0		19.9 ± 1.6		70.1 ± 4.8	
		AWD_{safe}	10.4 ± 0.9	ab	9.8 ± 0.9		20.2 ± 1.6		68.0 ± 4.3	
		AWD_{strong}	10.6 ± 1.0	a	10.4 ± 0.8		21.0 ± 1.6		69.7 ± 4.7	
Average	Selenio	WFL	10.9 ± 0.5	ab	10.0 ± 0.9		20.9 ± 1.3		72.9 ± 3.1	a
		AWD_{safe}	10.7 ± 0.7	b	10.1 ± 0.9		20.7 ± 1.4		68.8 ± 2.8	b
		AWD_{strong}	11.1 ± 0.6	a	10.7 ± 0.8		21.8 ± 1.3		71.5 ± 2.7	a
	Cammeo	WFL	10.4 ± 0.5	b	9.5 ± 1.1		19.9 ± 1.6		72.2 ± 4.2	a
		AWD_{safe}	11.0 ± 0.6	a	9.9 ± 0.9		20.9 ± 1.5		71.6 ± 3.0	a
		AWD_{strong}	11.2 ± 0.8	a	10.3 ± 1.0		21.5 ± 1.6		73.4 ± 2.6	a
	CL26	WFL	9.4 ± 0.3	a	9.4 ± 0.9		18.8 ± 1.1		65.1 ± 1.9	a
		AWD_{safe}	9.5 ± 0.5	a	9.5 ± 0.8		19.1 ± 1.2		63.6 ± 2.2	a
		AWD_{strong}	9.5 ± 0.5	a	10.2 ± 0.8		19.7 ± 1.2		64.2 ± 2.4	a
p(F)	WM		0.007		0.000		0.000		0.000	
	V		0.000		0.006		0.000		0.000	
	Y		0.020		0.000		0.000		0.001	
	WM × Y		ns		0.004		0.034		0.049	
	WM × V		0.013		ns		ns		0.046	
	V × Y		ns		ns		ns		0.002	
	WM × V ×Y		ns		ns		ns		ns	

[a]WFL: water seeding and continuous flooding; AWD_{safe}: water seeding and moderate AWD; AWD_{strong}: water seeding and severe AWD.

Table 2.3. Performance of the three water managements alone and in interaction with the two years and with the three varieties in terms of yield components (panicle density, spikelets per panicle, 1000 grain weight and sterility). Data are presented as average between N and N+ fertilization. Within each parameter, means followed by different letters denote differences among water managements within each year or variety (p(F)<0.05), while the absence of letters suggests no significant differences.

Year (Y)	Variety (V)	Water manag.[a] (WM)	Panicle density (m^{-2})		Spikelets ($panicle^{-1}$)		1000 grain weight (g)		Sterility (%)	
2021		WFL	656 ± 108	b	97 ± 10		29.6 ± 9.1	a	9.4 ± 2.1	
		AWD_{safe}	674 ± 121	a	101 ± 12		29.3 ± 8.9	ab	10.9 ± 2.7	
		AWD_{strong}	677 ± 116	a	103 ± 11		28.1 ± 8.6	b	11.6 ± 3.0	
2022		WFL	695 ± 142	a	86 ± 13		30.5 ± 9.4	ab	10.8 ± 2.0	
		AWD_{safe}	663 ± 103	b	84 ± 13		30.7 ± 9.9	a	11.9 ± 2.1	
		AWD_{strong}	666 ± 116	b	87 ± 11		30.3 ± 9.6	b	11.9 ± 2.7	
Average		WFL	675 ± 126		91 ± 13	b	30.0 ± 9.1		10.1 ± 2.2	b
		AWD_{safe}	668 ± 111		93 ± 15	ab	30.0 ± 9.3		11.4 ± 2.5	a
		AWD_{strong}	671 ± 115		95 ± 13	a	29.7 ± 9.1		11.7 ± 2.8	a
Average	Selenio	WFL	725 ± 54		92 ± 8		25.7 ± 0.7		9.4 ± 1.8	b
		AWD_{safe}	730 ± 53		92 ± 11		25.5 ± 0.7		12.9 ± 1.8	a
		AWD_{strong}	716 ± 55		94 ± 9		25.4 ± 0.7		12.2 ± 2.2	a
	Cammeo	WFL	518 ± 42		78 ± 8		42.6 ± 1.0		9.2 ± 2.0	a
		AWD_{safe}	538 ± 47		79 ± 10		42.8 ± 1.6		9.0 ± 2.1	a
		AWD_{strong}	530 ± 51		84 ± 9		42.1 ± 1.4		9.6 ± 2.4	a
	CL26	WFL	783 ± 61		103 ± 8		21.8 ± 0.3		11.8 ± 1.8	b
		AWD_{safe}	737 ± 82		106 ± 11		21.7 ± 0.3		12.3 ± 1.5	ab
		AWD_{strong}	768 ± 49		107 ± 11		21.5 ± 0.3		13.5 ±2.3	a
p(F)	WM		ns		0.006		0.000		0.000	
	V		0.000		0.000		0.000		0.000	
	Y		ns		0.000		0.000		0.006	
	WM × V		ns		ns		ns		0.002	
	WM × Y		0.026		ns		0.032		ns	
	V × Y		ns		ns		0.000		ns	
	WM × V ×Y		ns		ns		ns		ns	

[a]WFL: water seeding and continuous flooding; AWD_{safe}: water seeding and moderate AWD; AWD_{strong}: water seeding and severe AWD.

2.3.2. N uptake and apparent N recovery

Grain N contents were significantly affected by water management in interaction with year (Table 2.4). In 2021 higher values were found in WFL with respect to AWD_{strong} while AWD_{safe} showed intermediate values. In 2022 higher grain N contents were recorded in AWD_{safe} with decreasing values in AWD_{strong} and WFL. In general, straw N contents were significantly affected by water management, with the lowest values in both AWD treatments independently of the variety and year. In contrast, no water management-related differences were observed in total N uptake from both fertilized and control sub-sub-plots in both years and across all varieties. The supply of a higher amount of mineral N in the *N+* compared with the *N* treatment resulted in a significant increase in total N uptake in all water managements, although N content in grain and straw were not statistically affected by level of N applied. AWD_{safe} and AWD_{strong} slightly reduced the apparent N recovery (ANR) compared to WFL, although the differences were not significant. There is, however, a small effect of varieties on this parameter, with Cammeo showing a greater but not significant ANR under AWD compared to WFL, in contrast to the other varieties.

Table 2.4. Performance of the three water managements alone and in interaction with the two years, the three varieties and with two N fertilization treatments in terms of grain and straw N contents, total N uptake in fertilized and control plots, and apparent N recovery. Within each parameter, means followed by different letters denote differences among water managements within each year or variety or differences between N fertilization treatments within each irrigation (p(F)<0.05), while the absence of letters suggests no significant differences.

Year (Y)	Variety (V)	Water manag.[a] (WM)	Fert. (F)	Grain N (%)		Straw N (%)		Total N uptake (kg ha^{-1}) Fertilized		Control	Apparent N recovery (%)
2021		WFL		1.12 ± 0.13	a	0.58 ± 0.04		165.7 ± 18.0		97.2 ± 9.1	52.6 ± 13.4
		AWD_{safe}		1.06 ± 0.09	ab	0.53 ± 0.06		162.0 ± 16.2		100.5 ± 7.4	48.7 ± 10.5
		AWD_{strong}		1.04 ± 0.10	b	0.53 ± 0.07		163.8 ± 19.7		104.7 ± 9.4	46.4 ± 9.4
2022		WFL		1.13 ± 0.13	b	0.65 ± 0.11		184.0 ± 19.4		111.0 ± 10.5	57.9 ± 15.3
		AWD_{safe}		1.21 ± 0.14	a	0.60 ± 0.08		185.7 ± 25.1		113.4 ± 17.4	57.5 ± 13.1
		AWD_{strong}		1.19 ± 0.13	ab	0.57 ± 0.09		187.5 ± 16.5		115.2 ± 10.5	57.6 ± 15.5
Average		WFL		1.13 ± 0.13		0.62 ± 0.09	a	174.8 ± 20.7		104.1 ± 11.9	55.3 ± 14.7
		AWD_{safe}		1.14 ± 0.14		0.56 ± 0.08	b	173.8 ± 24.1		107.0 ± 14.6	53.1 ± 12.6
		AWD_{strong}		1.12 ± 0.14		0.55 ± 0.08	b	175.7 ± 21.6		110.0 ± 11.2	52.0 ± 13.9
Average	Selenio	WFL		1.06 ± 0.07		0.61 ± 0.08		177.5 ± 20.5		100.3 ± 8.1	64.7 ± 12.6
		AWD_{safe}		1.11 ± 0.13		0.56 ± 0.07		175.0 ± 25.0		109.3 ± 11.0	62.4 ± 11.6
		AWD_{strong}		1.07 ± 0.10		0.53 ± 0.09		175.9 ± 24.5		99.4 ± 11.9	55.2 ± 13.9
	Cammeo	WFL		1.11 ± 0.16		0.59 ± 0.05		171.1 ± 18.3		108.5 ± 8.8	49.2 ± 15.0
		AWD_{safe}		1.07 ± 0.12		0.55 ± 0.10		172.9 ± 28.9		109.9 ± 16.7	51.9 ± 12.2
		AWD_{strong}		1.04 ± 0.08		0.51 ± 0.05		169.1 ± 20.5		107.6 ± 13.1	51.3 ± 16.2
	CL26	WFL		1.21 ± 0.09		0.65 ± 0.12		176.0 ± 23.7		104.3 ± 16.7	51.6 ± 10.2
		AWD_{safe}		1.24 ± 0.11		0.58 ± 0.07		173.7 ± 19.0		110.6 ± 15.1	45.1 ± 7.2
		AWD_{strong}		1.24 ± 0.13		0.62 ± 0.07		182.1 ± 18.8		113.0 ± 8.9	49.6 ± 11.5
		WFL	N	1.10 ± 0.16		0.60 ± 0.07		164.9 ± 19.0	b		55.2 ± 17.5
			N+	1.15 ± 0.09		0.64 ± 0.10		184.8 ± 17.6	a		55.4 ± 11.7
		AWD_{safe}	N	1.11 ± 0.12		0.55 ± 0.08		162.4 ± 17.8	b		52.4 ± 11.7
			N+	1.17 ± 0.16		0.57 ±0.07		185.2 ± 24.5	a		53.8 ± 13.6
		AWD_{strong}	N	1.10 ± 0.16		0.54 ± 0.08		165.5 ± 19.9	b		52.1 ± 16.0
			N+	1.13 ± 0.12		0.56 ± 0.09		185.9 ± 18.5	a		51.9 ± 11.7
p(F)	WM			ns		0.000		ns		ns	ns
	V			0.000		0.000		ns		ns	0.000
	F			0.005		0.013		0.000		-	ns
	Y			0.000		0.000		0.000		0.000	0.000
	WM × V			ns		ns		ns		ns	ns
	WM × F			ns		ns		0.010		-	ns
	WM × Y			0.000		ns		ns		ns	ns
	F × V			ns		ns		ns		-	ns
	F × Y			ns		ns		ns		-	ns

[a]WFL: water seeding and continuous flooding; AWD_{safe}: water seeding and moderate AWD; AWD_{strong}: water seeding and severe AWD.

2.3.3. Metal(loid) grain concentrations

The adoption of AWD strongly affected the total grain content of metal(loid)s such as As, Cd and Ni (Table 2.5). Irrespective of the level of severity, lower concentrations of total As in the grain were observed under AWD. Also the influence of variety was relevant for As uptake. Although both AWD_{safe} and AWD_{strong} significantly reduced total As grain concentrations in Selenio and CL26 compared to WFL, concentrations in Cammeo grains were comparable across the three water managements. In contrast, Cd concentrations in the grain were significantly higher in plots managed with AWD than WFL, increasing in the order $WFL<AWD_{safe}<AWD_{strong}$. Higher concentrations of Cd were registered in 2022 than 2021 under both AWD_{safe} and AWD_{strong}. The effects of water management on grain Cd contents were similar across the three varieties, with CL26 showing an increasing trend in Cd contents with increasing AWD severity, whereas Selenio and Cammeo did not show significant differences between the two AWD treatments. As for Cd, Ni concentrations in the grain increased significantly with the adoption of both AWD treatments with respect to WFL, albeit without a significant interaction with rice variety.

Table 2.5. Grain concentrations of total arsenic (As), cadmium (Cd) and nickel (Ni) under the three water managements alone and in interaction with the two years and with the three varieties. Ni was monitored only in 2022. Within each parameter, means followed by different letters denote differences among water managements within each year or variety (p(F)<0.05), while the absence of letters suggests no significant differences.

Year (Y)	Variety (V)	Water manag.[a] (WM)	As		Cd		Ni	
			μg kg^{-1}					
2021		WFL	214.8 ± 74.4	a	16.2 ± 6.5	b		
		AWD_{safe}	173.8 ± 34.5	b	75.3 ± 39.5	b		
		AWD_{strong}	158.2 ± 41.5	b	165.0 ± 79.7	a		
2022		WFL	245.1 ± 42.6	a	18.9 ± 7.8	b	94.9 ± 48.4	b
		AWD_{safe}	149.2 ± 24.2	b	255.8 ± 73.4	a	492.0 ± 145.2	a
		AWD_{strong}	129.4 ± 21.2	b	309.3 ± 97.0	a	632.4 ± 268.0	a
Average		WFL	229.9 ± 61.2		17.3 ± 16.5		94.9 ± 48.4	b
		AWD_{safe}	161.5 ± 31.7		169.1 ± 109.1		492.0 ± 145.2	a
		AWD_{strong}	143.8 ± 35.4		237.1 ± 113.9		632.4 ± 268.0	a
Average	Selenio	WFL	254.1 ± 58.8	a	28.5 ± 23.8	b	71.7 ± 23.6	b
		AWD_{safe}	185.4 ± 32.5	b	140.1 ± 88.0	a	392.1 ± 76.7	a
		AWD_{strong}	147.3 ± 31.1	b	186.1 ± 84.0	a	503.1 ± 79.2	a
	Cammeo	WFL	184.9 ± 35.7	a	12.3 ± 6.3	b	60.3 ± 9.5	b
		AWD_{safe}	137.9 ± 14.0	b	185.7 ± 115.7	a	471.0 ± 143.3	a
		AWD_{strong}	150.5 ± 42.6	ab	249.2 ± 108.9	a	509.7 ± 189.2	a
	CL26	WFL	250.9 ± 63.3	a	11.0 ± 6.8	c	152.8 ± 34.2	c
		AWD_{safe}	161.1 ± 28.0	b	169.6 ± 131.3	b	612.9 ± 132.4	b
		AWD_{strong}	133.6 ± 33.3	b	276.1 ± 137.2	a	884.5 ± 306.8	a
p(F)	WM		0.000		0.000		0.000	
	V		0.003		ns		ns	
	Y		ns		0.000		-	
	WM × V		0.036		0.027		0.041	
	WM × Y		0.017		0.000		-	
	V × Y		ns		ns		-	

[a]WFL: water seeding and continuous flooding; AWD_{safe}: water seeding and moderate AWD; AWD_{strong}: water seeding and severe AWD.

2.3.4. Greenhouse gas emissions

Greenhouse gas emissions occurred throughout the entire cropping cycle and were strongly influenced by water management and soil reduction potential (Fig. 2.3 & 2.4). Similar measured redox potentials were recorded for all water managements up to the tillering stage; subsequently, higher redox potentials were recorded for the AWD treatments compared to WFL, where it dropped to negative values (Fig. 2.3). CH_4 fluxes reflected these changes in soil redox conditions as a function of water management. In both years and irrespective of water management, CH_4 fluxes were immediately observed in correspondence with the first week after seeding and increased rapidly showing a first major peak at the end of the "pin-point" period when flooding was restored (Fig. 2.4). Fluxes strongly decreased during the drainage periods performed to facilitate herbicide treatment and top-dressing fertilization at the tillering and panicle initiation stages, and after final field drainage before harvest. Before tillering CH_4 fluxes were similar in all three treatments due to the similar water management. After tillering, the introduction of AWD cycles significantly affected CH_4 fluxes, with a general reduction with respect to WFL, which was more pronounced in the later stages of crop development, particularly in 2022. After flooding, CH_4 emissions from WFL were rather high and relatively constant with highest emission peaks observed in early July, a few days before the panicle initiation stage, and a few days after the final drainage, in both years but particularly in 2021. On the other hand, under both AWD treatments, emissions tended to increase and decrease in correspondence with repeated field flooding and drainage during AWD cycles, with lowest fluxes measured for AWD_{strong}. Indeed, in 2022 during the reproductive and ripening stages, emissions under AWD were more constant and significantly lower than under WFL.

N_2O fluxes were relatively low over the two years across all water management practices except for a few significant peaks in correspondence with top-dressed mineral N fertilization events at tillering, although no relationship with water management was noted (Fig. 2.4). An additional important peak was recorded under AWD_{safe}

only in 2022 corresponding to the beginning of drainage operated at seedling stage for root anchoring. However, no other significant N_2O emissions were recorded under both AWD managements during the later stages of the cropping season, when AWD cycles could have promoted nitrification-denitrification.

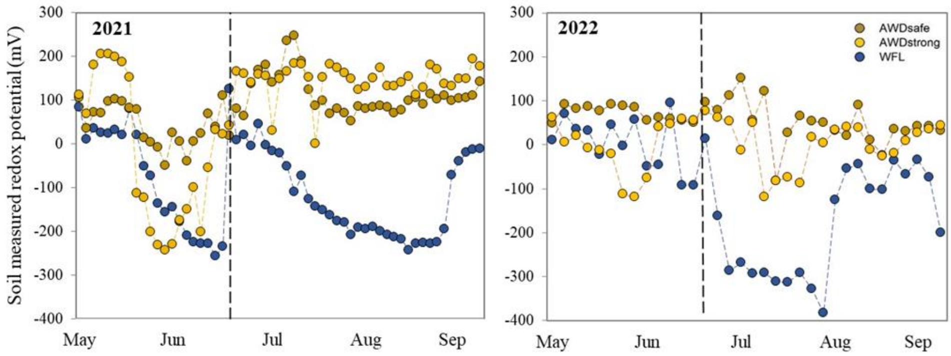

Figure 2.3. Seasonal variation in soil measured redox potential over two years (2021 and 2022) as a function of water management practices involving WFL (water seeding and continuous flooding), AWD$_{safe}$ (water seeding and moderate AWD) and AWD$_{strong}$ (water seeding and severe AWD). The dotted line represents the beginning of AWD cycles.

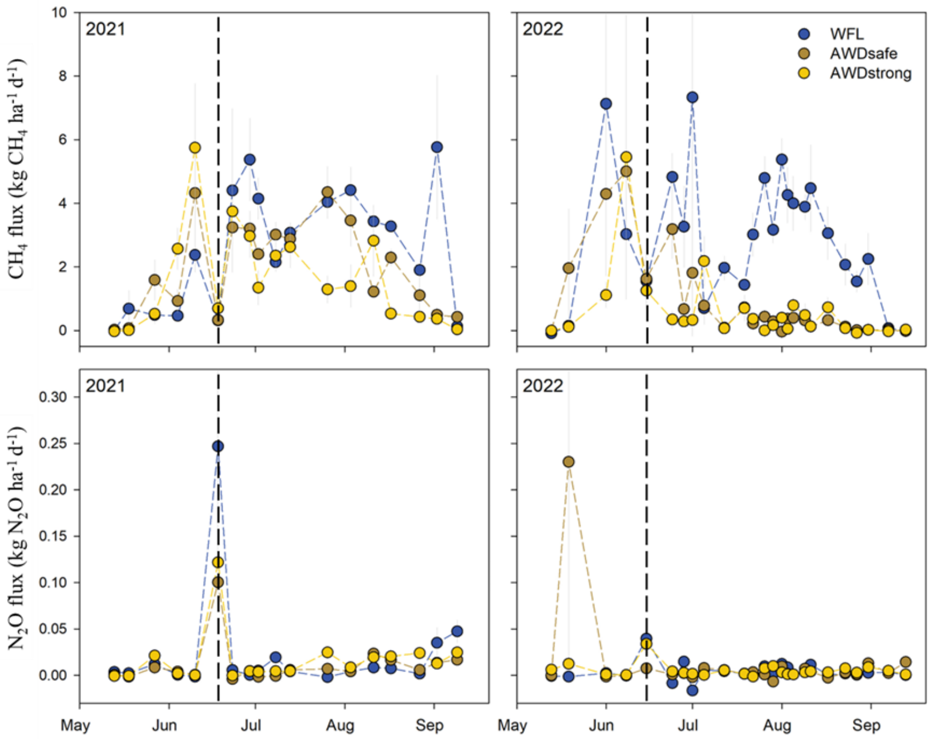

Figure 2.4. Seasonal variation in CH_4 and N_2O emissions fluxes over two years (2021 and 2022) as a function of water management practices involving WFL (water seeding and continuous flooding), AWD_{safe} (water seeding and moderate AWD) and AWD_{strong} (water seeding and severe AWD). The dotted line represents the beginning of AWD cycles.

In both years, adoption of AWD_{safe} and AWD_{strong} reduced cumulative CH_4 emissions with respect to WFL, although differences in 2021 were not statistically significant because of the high spatial variability of measured data (Fig. 2.5). Compared to total emissions of 352.4 and 347.4 kg CH_4 ha^{-1} under WFL in 2021 and 2022, adoption of AWD_{safe} and AWD_{strong} reduced total CH_4 emissions by 40-45 % and 55-73 %, respectively. However, a significant trend in CH_4 mitigation with increasing severity of AWD was only observed in 2022 where 92.3 kg CH_4 ha^{-1} total emissions under AWD_{strong} were measured. Cumulative N_2O emissions under WFL management were of 1.14 kg N_2O ha^{-1} in

2021, while in 2022 emissions were below the limits of quantification. In both years, no significant differences were observed in cumulative N_2O emissions with the adoption of AWD compared to WFL, irrespective of the severity (Fig. 2.5).

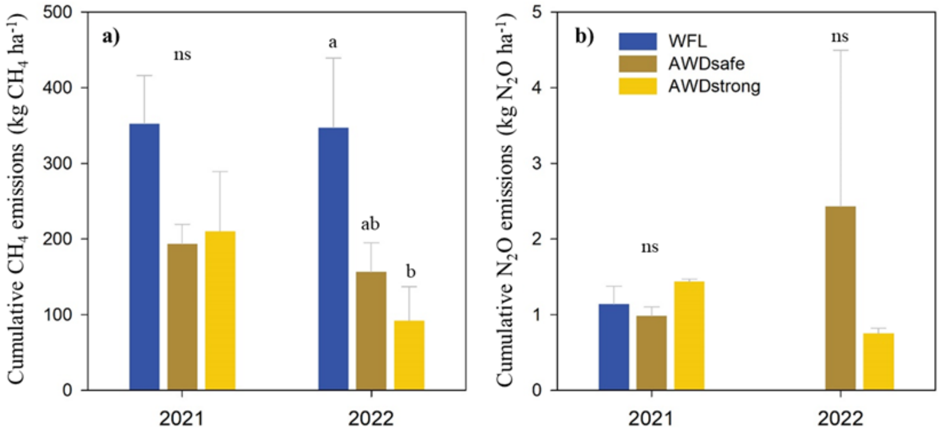

Figure 2.5. Cumulative emissions of CH_4 (a) and N_2O (b) over the cropping season for WFL (water seeding and continuous flooding), AWD_{safe} (water seeding and moderate AWD) and AWD_{strong} (water seeding and severe AWD) in both years. Measured N_2O emissions for WFL in 2022 were not quantifiable. Error bars represent the standard deviation of four replicates. Treatments p(F) was equal to 0.046 in 2022 for CH_4. Different letters represent significant differences among water managements within each year ($p(F)<0.05$).

Irrespective of the water management, CH_4 rather than N_2O was the main contributor to the GWP, accounting for 97-100% in WFL, 95-87% in AWD_{safe} and 94–93% in AWD_{strong} (Fig. 2.6). Considering the entire experimental period, AWD_{safe} and AWD_{strong} reduced the GWP by 46 and 54%, respectively, compared to WFL. Although the adoption of AWD consistently decreased the GWP, there was a large variability in the mitigation effect of the two AWD managements between the two years, particularly for AWD_{strong} that led to a reduction in the GWP of 71% in 2022 and only 38% in 2021, with respect to WFL. GHG Eco-efficiency increased in the order WFL < AWD_{safe} < AWD_{strong} in both years, but significant differences were only found in 2022, where AWD_{strong} showed highest values for this index while WFL and AWD_{safe} did not differ substantially (Fig 2.6).

Mean EF calculated for CH_4 and expressed as kg CH_4 ha^{-1} d^{-1}, showed significantly lower values with the adoption of AWD with respect to WFL, although differences between the two severities of AWD were not significant (Table 2.6).

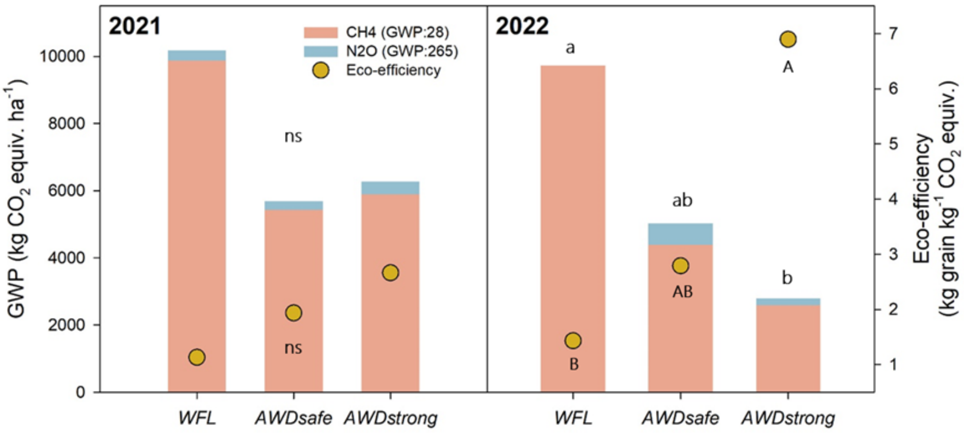

Figure 2.6. GWP (Global Warming Potential) as sum of N_2O and CH_4 and Eco-Efficiency for WFL (water seeding and continuous flooding), AWD_{safe} (water seeding and moderate AWD) and AWD_{strong} (water seeding and severe AWD) in the 2021 and 2022 cropping seasons. Treatments p(F) was equal to 0.041 and 0.039 in 2022 for CH_4 and N_2O, respectively. Different lowercase and capital letters represent significant differences among treatments in GWP and Eco-Efficiency, respectively ($p(F)<0.05$).

Table 2.6. Annual and mean emission factor for CH_4 in the three water managements. Means followed by different letters within each year denote differences among water managements (p(F)<0.05), while the absence of letters suggests no significant differences.

Water management[a]	CH_4 emission factor 2021 (kg CH_4 ha^{-1} d^{-1})	CH_4 emission factor 2022 (kg CH_4 ha^{-1} d^{-1})		Mean CH_4 emission factor (kg CH_4 ha^{-1} d^{-1})	
WFL	2.43	2.63	a	2.54	a
AWD_{safe}	1.34	1.20	ab	1.27	b
AWD_{strong}	1.45	0.70	b	1.08	b
p(F)	ns	0.045		0.008	

[a]WFL: water seeding and continuous flooding; AWD_{safe}: water seeding and moderate AWD; AWD_{strong}: water seeding and severe AWD.

2.4. Discussion

2.4.1. Rice productivity

The adoption of AWD is often accompanied by variable yield gaps with respect to conventional water management mainly due to changes in plant phenology (e.g. root development), tolerance to water stress, and nutrient uptake by plants (Miniotti et al., 2016; Volante et al., 2017; Zhang et al., 2009). Frequent changes in soil redox status are also known to influence a variety of processes controlling N distribution, transformation, losses, and consequently, bioavailability for rice (Cucu et al., 2014; Said-Pullicino et al., 2014), that could have important effects on rice productivity. All these confounding factors are probably responsible for the different effects of AWD on grain yields reported in literature, that vary from lower to higher yields with respect to continuous flooding. Several authors reported no yield gaps when AWD with a soil water potential threshold of around –5/–10 kPa was adopted (i.e. AWD_{safe}) in temperate rice cropping systems (Carrijo et al., 2018; Monaco et al., 2021; Runkle et al., 2018), while others noted significant losses in grain yields when more severe AWD cycles (down to –20 kPa) were adopted, especially in light textured soil (Ishfaq et al., 2020), or when AWD was applied in conjunction with dry seeding over the whole cropping season (Carrijo et al., 2017; Miniotti et al. 2016), or when rice varieties less tolerant to AWD were grown (Martínez-Eixarch et al., 2021). In this study we evidenced similar or higher grain yields under AWD with respect to WFL. Nonetheless, the tested varieties had a different adaptability to AWD with Cammeo obtaining the highest yield gain with respect to conventional water management. These results are in line with the minor effects of mild AWD on the grain yields of different European rice cultivars tested in Italy (Monaco et al., 2021). The different levels of severity in AWD_{safe} and AWD_{strong} did not result in different grain yields, with the exception of Selenio, for which the observed differences were not related to different yield component responses to AWD. This indicates that AWD_{strong} was not the threshold level in this study, and more severe levels could presumably be applied without incurring in yield losses. We speculate that the good performance of rice under both safe and strong AWD in water seeded rice was probably due to the loamy soil texture of our study site that allowed for good root establishment, limited water stress during dry periods, and the lower incidence of physiological stresses typically related to the reducing conditions of continuous flooding, such as nutritional disorders (e.g. Akiochi), caused by sulfides, reduced iron, and volatile fatty acids, which can lead to early crop decline and lower nutrient uptake, especially in the reproductive stage with negative impacts on productivity (Pan et al., 2009). Furthermore, a higher incidence of stem rot of rice (*Sclerotium oryzae* Catt.) was observed under WFL (data not shown), especially in Cammeo, probably responsible for the lower grain yield respect to AWD.

The variability in grain yield among different rice varieties under different water managements highlighted in this study suggests the need for further investigation to identify the phenotypic characteristics that endow rice varieties with a better adaptability to water stress under AWD.

AWD_{safe} reduced plant height compared to continuous flooding, as also observed by Norton et al. (2017a) and Santiago-Arenas et al. (2021), despite the similar straw yield and total biomass. Our results that panicle density and 1000 grain weight were not affected by water management, also in interaction with variety, are also confirmed by the findings of Monaco et al. (2021) and Norton et al. (2017a). Higher yield potential under AWD was attributed to a higher number of spikelets per panicle, as already observed by Chu et al. (2018) and Yushi et al. (2013), despite higher sterility in all studied cultivars except Cammeo, related to a water-deficit stress that probably occurred during flowering (Pascual and Wang, 2017).

Water management can also strongly affect nutrient availability for plant uptake. Several studies have reported that the frequent alternation between field flooding and drainage during AWD cycles may promote N losses as N_2O and N_2 emissions during nitrification/denitrification processes and nitrate leaching, and enhance microbial N immobilization, thereby contributing to a lower N availability for plant uptake and consequently lower nutrient use efficiency (Cucu et al., 2014; Dong et al., 2018; Li et al., 2018; Shekhar et al., 2021). On the other hand, improved soil aeration under AWD may accelerate organic matter (and organic N) mineralization and promote belowground C allocation by plants as their roots explore deeper soil layers for enhancing nutrient uptake and consequently grain and biomass yield (Dong et al., 2012;

Kato and Katsura, 2014; Zhang et al., 2009). In line with other studies (Carrijo et al., 2018; Cheng et al., 2022; Ye et al., 2013), total plant N uptake was not affected by water management even though straw N content was slightly but significantly lower under AWD with respect to WFL. We also observed a slight but not significant reduction in ANR with both AWD_{safe} (53%) and AWD_{strong} (52%) compared to continuous flooding (55%), in line with the findings of Cheng et al. (2022) and Pan et al. (2017).

Vitali et al. (2024) have recently shown that AWD can influence the contribution of different N sources to plant uptake, not only by resulting in a slightly lower fertilizer-N use efficiency due to higher losses (Chu et al., 2015; Wang et al., 2016), but also by decreasing the soil N supply with respect to continuous flooding. However, they also show that these effects also depend on the management of crop residues and timing of fertilizer N application in relation with water management. In our study, the minimal differences between the two AWD regimes were probably due to a correct management of fertilizer application and irrigation management by which field flooding was carried out immediately after N application thereby minimising N losses (Lampayan et al., 2015; Yang et al., 2017). Irrespective of water management, the tested varieties showed significantly different ANR with Selenio showing a higher N recovery (on average 60.8 %) than Cammeo and CL26 (50.8 and 48.8 %, respectively), suggesting that varietal selection plays an important role in the management of N use efficiency under different water management practices. The specific root systems of the varieties, together with the greater root growth and activity under AWD (Islam et al., 2020a), may have influenced nutrient absorption capacity and consequently ANR by the different varieties.

2.4.2. Grain quality / Metal(loid) grain concentrations

Total grain As concentrations under continuous flooding were on average 230 µg kg^{-1}, which is similar to those reported by Monaco et al. (2021), but lower than values reported by Linquist et al. (2015). AWD_{safe} and AWD_{strong} reduced As concentration by 30 and 37%, respectively, compared with continuous flooding, in line with results observed in other studies across Europe (38-40%) (Martínez-Eixarch et al., 2021; Monaco et al., 2021). In contrast, adoption of AWD_{safe} in fine textured paddy soils in California did not diminish grain As contents because reducing soil conditions persisted even during dry periods after drainage due to a higher water retention (Carrijo et al., 2018). In our experiment, the loamy soil allowed for a rapid increase in measured redox potential immediately after field drainage, thereby reducing As concentration in the soil solution via coprecipitation/adsorption with Fe oxy(hydr)oxides (Zecchin et al., 2017). AWD_{strong} showed a slightly higher potential to reduce As accumulation than AWD_{safe}. Although the differences observed in this work were not significant, this trend corroborates the findings of Linquist et al. (2015) and Carrijo et al. (2018, 2019, 2022), who assessed a direct relationship between the severity and number of periods of field drainage during AWD cycles and the decrease of grain As concentration. We also observed a significant varietal effect, in line with previous studies (Tenni et al., 2017). Moreover, the tested varieties responded differently to water management in terms of As uptake, as reported for tropical rice varieties (Norton et al., 2017b). In fact, Cammeo showed the lowest grain As content under WFL among all varieties, but AWD practices had the least beneficial effect in decreasing As uptake. However, altogether, the reductions in total As content achieved with

AWD in our study show that this water management represents a valuable tool for keeping As concentration in rice grain within the legal limit stated by the European Commission for inorganic As.

As expected, the effects of water management on grain Cd contents had an opposite trend with respect to As, as the adoption of AWD resulted in 10- to 13-times higher Cd contents compared to continuous flooding. Monaco et al. (2021) reported grain Cd concentrations of 135 $\mu g\ kg^{-1}$ with AWD_{safe}, which is lower than the 169 and 237 $\mu g\ kg^{-1}$ measured in our experiment under AWD_{safe} and AWD_{strong}, respectively. This could be attributable to the application, in our work, of AWD drying periods during the flowering and ripening stages, which are known to increase Cd mobility in soil during the phenological stages at which the greatest Cd translocation towards the grain occurs (Carrijo et al., 2022). In contrast to As, no significant varietal effect for Cd uptake was observed, while the effect of the different climatic conditions characterizing the two years of our experiment was evident. The drier summer in 2022 probably favoured Cd mobilization because of a faster decrease in soil moisture, involving rapid changes in soil redox potentials and pH, while the higher temperatures (Fig.1) may have increased plant transpiration and thus Cd uptake and translocation to the grain (Cattani et al., 2008). Under both AWD managements, Cd grain contents exceeded the 150 $\mu g\ kg^{-1}$ limit imposed by the European Union (Commission regulation, 2021), thereby confirming a critical water management-related trade-off between As and Cd grain contents, with important implications for food safety and human health. The management of AWD (severity, timing and number of soil drying periods) has been shown to be more critical for Cd than for As (Carrijo et al., 2022), hence, the best trade-off between As and Cd uptake could be achieved implementing AWD cycles during those phenological stages when rice is less sensitive to Cd accumulation, taking advantage of the beneficial effect of soil drying at stem elongation for As decrease (Zecchin et al., 2017), while keeping the soil flooded during the flowering stage to reduce Cd uptake (Carrijo et al., 2022).

Although Ni concentration was only investigated in 2022, our results evidenced that the adoption of AWD also led to an important increase in grain Ni content (5- to 7-time higher) compared to continuous flooding. The varietal effect was the same observed for Cd and indeed, while Ni and Cd concentrations in rice grain were positively related, both contaminants were inversely related with respect to As. However, while the different mechanisms linking As and Cd release from the soil solid phases to porewater in redox-fluctuating environments and the consequent uptake by rice plants are quite well understood, the same cannot be said for Ni, since the concentration of this element in soil solution is generally enhanced under reducing conditions (Rinklebe and Shaheen, 2017), even though our results corroborate the increasing evidences that more oxidizing conditions favour the accumulation of Ni in the rice grain (da Silva et al., 2020; Norton et al., 2017b; Orasen et al., 2019). Further studies are thus needed to better elucidate the apparent decoupling between Ni solid/solution partitioning in paddy soils and its accumulation in rice as a function of changing redox potentials and pH, in order to contrast this adverse effect with the application of AWD.

2.4.3. Greenhouse gas emissions

Total methane emissions over the cropping season under conventional water management (347-352 kg CH_4 ha^{-1}) were in line with values reported by Bertora et al. (2018b) and Peyron et al. (2016) for similar cropping systems in the region where rice was water seeded, paddy fields were continuously flooded and crop residues were incorporated more than 30 d before seeding. Moreover, our results confirmed that AWD significantly reduced cumulative CH_4 emissions particularly when lower soil water potentials were reached with the adoption of more severe AWD thresholds, even though these trends were stronger and more significant in the drier year (i.e. 2022). Mitigation of CH_4 emissions by AWD is generally due to the more aerobic soil conditions during the cropping season that are known to inhibit methanogenesis and favour aerobic decomposition and mineralization of labile organic matter, with respect to the conventional continuous flooded practice (Said-Pullicino et al., 2016), thereby resulting in substantially lower mean fluxes. However, continuous flooding also led to the production of high emission peaks in correspondence with field drainage that contributed substantially to the total cumulative emissions, but that were not observed under AWD. Similar peaks have been reported elsewhere (Linquist et al., 2015; Peyron et al., 2016) and have often been attributed to the rapid loss of entrapped CH_4 during field drainage (Pittelkow et al., 2013; Runkle et al., 2018). The absence of this phenomenon under AWD management was probably due to the higher soil redox potentials that limited production and accumulation of entrapped CH_4 in soil pores (Linquist et al., 2015).

The effectiveness of AWD to reduce total CH_4 emissions with respect to WFL (by 40-45 % and 55-73 % with AWD_{safe} and AWD_{strong}, respectively herein) are in line with the mitigation effects reported by Lagomarsino et al. (2016), LaHue et al. (2016), Martínez-Eixarch et al. (2021) for temperate rice systems, even though reductions in excess of 90 % were often observed when AWD management was combined with dry seeding and delayed flooding (Lagomarsino et al., 2016; Linquist et al., 2015; Peyron et al., 2016) or winter flooding (Martínez-Eixarch et al., 2021). These latter practices allow for a better aerobic decomposition of crop residues before the beginning of the cropping season that further reduces the amount of labile organic substrates for methanogens after flooding (Said-Pullicino et al., 2016).

Although the potential of AWD to mitigate CH_4 emissions from water seeded temperate rice paddies is evident and clearly related to AWD severity, the extent to which AWD can contribute to the mitigation with respect to conventional practices is highly variable (both spatially and temporally) and strongly depends on the interacting effects of pedoclimate, water availability and land suitability, that still remain hard to elucidate. The spatial variability may be related to different soil permeability properties and soil redox conditions, which are key aspects in influencing GHG emissions under AWD (Cheng et al., 2022). Similarly, the punctual management of water levels in the field for the correct adoption of AWD strongly depends on irrigation water availability, meteorological and hydrological conditions, that may all differ substantially between cropping seasons.

According to the guidelines of Intergovernmental Panel on Climate Change (IPCC), CH_4 emissions from rice paddies can be best estimated by utilizing country-specific daily emission factors (EF) and scaling factor (SF_w), which is a value calculated for different water management practices relative to continuously flooded fields (IPCC, 2019, Chapter 5.5). By adopting the IPCC Tier 1 approach for the estimation of CH_4 emissions from rice paddies, the daily EF for AWD can be estimated by multiplying the default CH_4 baseline EF for continuously flooded rice cultivation in Europe that ranges between 1.06–2.31 kg CH_4 ha^{-1} d^{-1} by the SF_w for multiple drainage periods during the rice cropping season (i.e. AWD) of 0.55, resulting in an EF that ranges between 0.58–1.27

kg CH_4 ha^{-1} d^{-1}. On the basis of the data provided herein we calculated a mean daily EF for AWD of 1.18 kg CH_4 ha^{-1} d^{-1} over the rice cropping season. This would equate to a SF_w of 0.46 when considering an EF for WFL measured in this study of 2.54 kg CH_4 ha^{-1} d^{-1}. Alternatively, a SF_w of 0.48 with an error range of 0.29 – 0.60 resulted when an aggregated mean EF for CH_4 emissions of 2.45 kg CH_4 ha^{-1} d^{-1}, that includes data from other water seeded, continuously flooded managements in the area, is considered (Peyron et al. 2016; Bertora et al. 2018b). The mean mitigation potential of AWD measured in this work is slightly higher than the 45% reduction for multiple drainages proposed by the IPCC Tier 1 methodology and should therefore be preferentially used for improving the estimation of CH_4 emissions from Italian rice paddies according to a Tier 2 approach (IPCC, 2019).

The adoption of AWD in rice paddies is often associated with a trade-off between CH_4 and N_2O emissions, as frequent field drainage and re-flooding cycles intended to mitigate CH_4 emissions, may enhance N_2O emissions by favouring denitrification/nitrification and decreasing N_2O reduction, particularly in the days following N fertilizer application (Lagomarsino et al., 2016; Miniotti et al., 2016; Verhoeven et al., 2018). These emission peaks have been shown to be strongly linked to crop development, and the integrated management of N fertilization and subsequent field flooding (Islam et al., 2020c; Kreye et al., 2007). In fact, as previously reported by Peyron et al. (2015), we measured highest N_2O emissions in correspondence with field flooding after N fertilization during the early vegetative stages, while N fertilization at the panicle initiation stage did not result in significant N_2O fluxes, probably because of the rapid N assimilation by rice plant in active growth (Hashim et al., 2015). Contrary to many previous studies, the adoption of AWD in our study did not increase cumulative N_2O emissions probably due to a careful water management in the days immediately following N fertilizer application. It was previously shown by Linquist et al. (2015) that reflooding the field within 24 h after the top-dressing fertilizer distribution, and maintaining flooding conditions for 7-10 days after fertilization allow maximum N uptake by the crop. This limits the amount of N available for nitrification/denitrification processes during dry periods, contributing to minimize N_2O emissions.

As already highlighted by various studies (Fertitta-Roberts et al., 2019; Islam et al., 2020b; Mazza et al., 2016), CH_4 emissions accounted for a substantial part of the GWP compared to N_2O emissions (99% in continuous flooding and 93% on average in the two AWD managements). Consequently, N_2O emissions only had a slightly higher weight in the GWP of AWD than in continuous flooding (7% on average in the two AWD managements and 1% in continuous flooding). The trade-off between CH_4 and N_2O emissions under AWD was previously shown to result in either lower GWP (Linquist et al., 2015; Mazza et al., 2016, Martínez-Eixarch et al., 2021) or higher GWP (Lagomarsino et al., 2016; Liao et al., 2020) compared to continuous flooding. In our study, reduced CH_4 emissions and similar N_2O emissions under AWD resulted in an overall reduction in the GWP of the cropping systems compared to conventional continuous flooding. As for CH_4 emissions, the GWP decreased with increasing AWD severity, by 46 and 54% on average for AWD_{safe} and AWD_{strong} with respect to continuous flooding, respectively, while the Eco-efficiency increased by 49-79% (in 2022), confirming the higher agro-environmental performance of AWD managements in line with the findings of Miniotti et al. (2016).

2.5. Conclusions

This study confirms that the environmental impact of conventional continuous flooding in Italian temperate rice systems can be mitigated through the adoption of AWD while maintaining similar or improved agronomic performance. The higher yield potential under AWD is determined by a higher number of spikelets per panicle, despite higher sterility, balanced by similar plant N uptake compared to continuous flooding. The variability in grain yields among different rice varieties suggests the need to identify genotypes more suitable for AWD. AWD treatments applied in combination with water seeding allow to significantly reduce CH_4 emissions without increasing N_2O emissions, thereby maintaining a lower GWP. The most important insight of this work is that the improvement in Eco-efficiency increased with the severity of AWD management when applied from tillering to maturity, without affecting yield and N uptake. Despite these potential benefits, our results also showed that there are important trade-offs related to food safety that need to be taken into consideration when adopting AWD. In fact, although AWD was found to be an appropriate strategy to reduce rice grain As concentrations, a contemporary increase in Cd and Ni contents may be of concern and requires specific abatement measures. Further studies needed to promote the adoption of AWD in temperate rice cropping systems should focus on the pedoclimatic and hydrological suitability of different rice farming areas (or hydrological districts) for AWD adoption, as well as on the most appropriate methods for implementing specific AWD thresholds (i.e. timing of drainage and reflooding cycles). The interannual variability in the GHG mitigation potential of AWD compared to conventional water management also represents an important limitation that needs further investigation in order to facilitate the diffusion of AWD not only in Italy, but also in Europe and other temperate rice-growing areas.

3. Interaction between water, crop residue and fertilization management on the source-differentiated nitrogen uptake by rice

3.1 Introduction

Rice cultivation with conventional continuous flooding (CF) requires large amounts of water, with the production of 1 ton of rice grain requiring approximately 2500 tons of water (Bouman et al., 2009). Moreover, water for agriculture is becoming increasingly scarce, due to climate change-related variations in rainfall patterns, decreasing resources and quality, inefficient irrigation systems and competition from other sectors such as urban and industrial users (Bouman et al., 2007b). Adequate availability of water resources to sustain crop yields is thus one of the most pressing challenges rice cropping systems are currently facing (Arcieri and Ghinassi, 2020).

In recent decades, the alternate wetting and drying (AWD) technique, in which fields are drained and re-flooded one or more times during the growing season, has become one of the most widespread water-saving irrigation technologies in paddy field (Song et al., 2020). AWD saves irrigation water by up to 38% (Shao et al., 2015; Carrijo et al., 2017; Song et al., 2021) and contributes to the reduction of CH_4 emissions, lowering the global warming potential with respect to CF (Li et al., 2018; Malumpong et al., 2021), while maintaining or even improving yields (Lampayan et al., 2015; Lahue et al., 2016; as well as results presented in Chapter 2).

However, the change of water management from CF to AWD may influence soil nutrient cycling and the functioning of soil microorganisms

(Cao et al., 2022). Indeed, Yang et al. (1999) pointed out that drying and re-wetting cycles in AWD affect biochemical and physical processes, namely, nitrification, denitrification, mineralization, percolation and leaching in soil by changing soil water and air equilibrium, which in turn affects N availability for plant uptake. The effects of AWD on the N dynamics in the soil–plant system and on N use efficiency (NUE) have been widely investigated but giving contradictory findings. Several studies have reported that the alternation between aerobic and anaerobic soil conditions in AWD may promote nitrification and denitrification responsible for enhanced production of N_2O (nitrification and partial denitrification) or N_2 (total denitrification), as well as NO_3^- leaching and NH_3 volatilization, resulting in substantial N losses, lower plant N uptake and consequently a lower NUE (Tan et al., 2015; Miniotti et al., 2016; Dong et al., 2018; Li et al., 2018; Jin et al., 2020; Lopez-Aizpun et al., 2021; Cheng et al., 2022). On the contrary, some studies demonstrated that there was no increase in N losses or even an enhancement of NUE with the adoption of AWD (Liu et al., 2013b; Yang, 2015; Wang et al., 2016; Djaman et al., 2018; Hameed et al., 2019; Islam et al., 2022). In addition, an improved soil aeration with AWD can accelerate organic matter mineralization enhancing the net release of available N for plant uptake from both soil organic matter (SOM) and incorporated crop residues (Zhang et al., 2009; Dong et al., 2012; Cucu et al., 2014; Chu et al., 2015; Fang et al., 2018). Thus, there is an increasing need to study the effects of AWD on NUE to optimize fertilizer-N management and reduce negative environmental impacts (Cheng et al., 2022; Wang et al., 2022).

Understanding the influence of water management on N availability for rice growth have always been influenced by the different effects that water management can have on the fate of applied N fertilizers and crop residue-derived N as well as native sources of soil organic N that are all known to contribute to plant nutrition (Said-Pullicino et al., 2014; Akter et al., 2018). Several studies have focused on evaluating the contribution of different N sources to rice nutrition under both CF and AWD conditions (Pan et al., 2012; Chen et al., 2016; Zhou et al., 2020); however, knowledge about the relative contribution of all three sources of N (fertilizer, crop residues and soil) as a function of water management is still poorly understood.

Crop residues and N fertilization management that are highly accountable for rice nutrition and for driving N availability in paddy soils are strongly coupled to water management practices (Bird et al., 2003; Kogel-Knabner et al., 2010; Said-Pullicino et al., 2014). It is well-known that poor N fertilizer use efficiency (30–40% recovery of applied N) occurs in continuously flooded rice systems (Cassman et al., 2002), with the remainder of total N uptake by rice derived from native soil N (Reddy, 1982; Cassman et al., 1998). Under periodic flooding and drying conditions, SOM and its redox properties play a crucial role in driving microbial processes that influence N availability for plant uptake and N losses (Nie et al., 2023). The release of N for plant uptake during straw decomposition depends on the balance between microbial N mineralization and immobilization, in turn affected by the availability of labile C sources for microbial activity and soil redox conditions (Nannipieri and Paul, 2009). Moreover, both crop residue and water management practices adopted in rice cropping systems may strongly influence microbial and abiotic immobilization of applied fertilizer-N and consequently N availability for rice. In particular, lower soil redox conditions and the addition of labile organic matter may enhance N immobilization (27–50% of applied fertilizer-N under flooded conditions) (Devevre and Horwath, 2000; Said-Pullicino et al., 2014) that may be subsequently released in time, contributing to available N for plant uptake (Devevre and Horwath, 2001; Nannipieri and Paul, 2009). Besides immobilization, the combination of crop residue and flood water management practices may strongly affect N losses from paddy fields representing between 10 and 65% of applied fertilizer-N (Cassman et al., 1998; Ghosh and Bhat, 1998). Therefore, temporal synchrony between fertilizer, crop residue and indigenous N supply and plant uptake is a crucial factor in determining N use efficiency (Cucu et al., 2014).

This work aims to provide insights into how AWD affects N cycling in paddy soils and the contribution of different N sources to plant nutrition, with respect to CF practices, as a function of crop residue incorporation and mineral N fertilization. Although various studies have focused on the interactions between water, fertilizer and crop residue management on the availability and plant uptake of N in rice paddies, this study evaluates the effects of the interactions between water management and the timing of crop residue and fertilizer application on soil processes driving the partitioning between different N sources in their contribution to total plant N uptake. These insights are important in order to identify suitable fertilization practices that favour plant N uptake and minimize N losses particularly during the early stages of rice growth under AWD when most N losses in the field are recorded (Miniotti et al., 2016). We hypothesized that:

(1) **AWD will decrease the contribution of fertilizer derived N to plant N due to increased N losses but increase N derived from crop residue and SOM mineralization due to the faster degradation under oxic soil conditions;**

(2) **anticipating the incorporation of rice straw with respect to soil flooding can promote their mineralization (and release of straw-N for plant uptake) under aerobic soil conditions and limit the immobilization of fertilizer-N during the growth period;**

(3) **increasing the amount of fertilizer-N applied at seeding and reducing the amount applied at tillering (with the start of AWD cycles) can improve N supply by temporarily enhancing N immobilization by the microbial biomass and limiting the losses of fertilizer-N during redox cycling and stimulate the metabolic degradation of organic matter and release of soil- and straw-derived N (i.e. priming).**

We tested these hypotheses by a mesocosm experiment in which rice was grown for 60 days (vegetative stage) in a growth chamber, under two different water regimes (CF vs. AWD). The paddy soil received both mineral and organic N (in the form of rice straw). Changes in plant-available N and microbial biomass, plant development and N uptake during the growth period were followed. We adopted a dual-stable isotope ^{15}N tracing approach to partition plant N between fertilizer-derived, straw-derived or indigenous N, in order to identify those sources and processes that have a major influence on N availability and plant nutrition.

3.2. Materials and methods

3.2.1. Soil and straw properties and mesocosm design

Soil was collected from the Ap horizon (0–15 cm) of a paddy soil (Haplic Gleysol) located within the Rice Research Centre of Ente Nazionale Risi at Castello d’Agogna (45°14′48″N, 8°41′52″E, NW Italy). The field has been under continuous rice cultivation for the last 30 years, with crop residue incorporation in spring, and field flooding for most of the cropping period (May to September). Soil was collected at the end of the cropping season (October) after removal of straw on the surface. The collected soil was air dried and sieved at 5 mm. The main physicochemical properties of the soil are organic C, 11.8 g kg^{-1}; total N, 1.3 g kg^{-1}; pH, 5.9; CEC, 9.4 cmol kg^{-1}; exchangeable Ca^{2+}, Mg^{2+} and K^{+}, 38.5, 12.6 and 20 mg kg^{-1}, respectively; P Olsen, 20.1 mg kg^{-1}; clay, 101 g kg^{-1}; silt, 463 g kg^{-1}; and sand, 437 g kg^{-1}. Rice straw was obtained from a previous field experiment in the same experimental platform, in which rice plants were repeatedly labelled with isotopically enriched N fertilizer (urea, 2.000 atom% ^{15}N) to ensure uniform enrichment of the straw at harvest and that had an isotopic enrichment of 0.925 atom% ^{15}N. Non-enriched rice straw was sampled from the same field from plots that received natural abundance urea fertilizer. The total N contents of non-enriched and ^{15}N enriched straw were 6.1 g N kg^{-1} and 5.7 g N kg^{-1}, respectively, while the total C contents were 372 g C kg^{-1} and 362 g C kg^{-1}, respectively.

Mesocosms were built using a cylindrical polyvinyl chloride (PVC) pipe (75 mm inner diameter, 350 mm height), whose bottom was closed with a non-woven fabric to allow for changes in water potentials during flooding and drainage periods (Fig. 3.1). Each mesocosm was filled with 1 kg of soil (oven dry basis) and placed inside a bucket (300 mm diameter, 400 mm height) containing 5 cm of gravel at the bottom on which the mesocosms rested, in order to allow water management during flooding and drainage periods. Changes in soil water potential inside the mesocosms during the experiment were controlled by regulating the water level in the buckets: flooding was carried out by introducing water into the bucket until the water level in the mesocosm and bucket were both around 3 cm above the soil level, while drainage was managed by reducing the water level in the bucket to the desired level through a plastic drain pipe. The mesocosms were located inside a growth chamber during the whole experimental period.

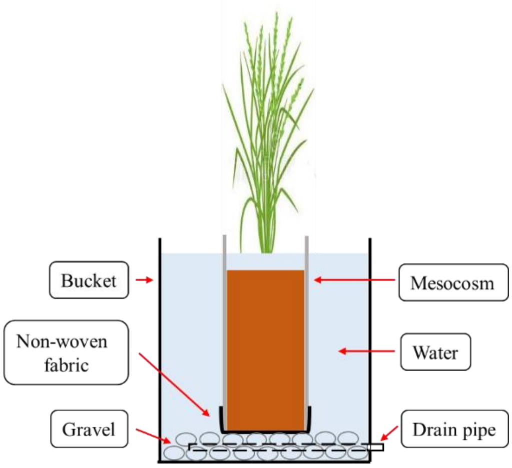

Figure 3.1. Representation of mesocosm and system adopted for water management in the experiment.

3.2.2. Experimental design

The experimental design comprised a completely randomized 2 × 3 factorial arrangement with water management as the main factor (continuous flooding (CF) vs alternate wetting and drying (AWD)) and three combinations of straw and fertilizer management as the second factor (Table 3.1). The three treatments differed in the timing of straw incorporation with respect to seeding (30 vs 60 days before seeding; S30 and S60, respectively) and in the splitting of applied fertilizer-N (52.8 mg N kg^{-1} soil, equivalent to 120 kg N ha^{-1}, considering a mesocosm area of 44 cm^2) between pre-seeding and tillering stage

(60 + 60 vs 80 + 40 kg N ha^{-1}; N60-N60 and N80-N40, respectively). S30-N60-N60 is the conventionally applied treatment in the Italian rice cultivation system, while S60-N60-N60 and S30-N80-N40 allowed to test hypothesess 2 and 3, respectively.

Table 3.1 Details of the differences in water, straw and fertilization management between the different experimental treatments.

Water management[a]	Treatment	Straw incubation (days before seeding)	N fertilization (kg N ha^{-1})	
			Pre-seeding	Tillering
CF	S30-N60-N60	30	60	60
	S30-N80-N40	30	80	40
	S60-N60-N60	60	60	60
AWD	S30-N60-N60	30	60	60
	S30-N80-N40	30	80	40
	S60-N60-N60	60	60	60

[a] CF: continuous flooding; AWD: alternate wetting and drying

Each of the three treatments was replicated in 9 mesocosms, in order to obtain 27 mesocosms for each water management. To investigate the different contribution of fertilizer-, straw- and soil-derived N to plant nutrition, ^{15}N-labelled materials were used in dedicated mesocosms. Three of the 9 replicated mesocosms for each treatment previously described received ^{15}N-labelled straw and natural abundance fertilizer (15SN), and another three received natural abundance straw and ^{15}N-labelled fertilizer (S15N), while the last three received natural abundance straw and natural abundance fertilizer (SN). Mesocosms were destructively sampled at 60 DAS for both CF and AWD, while an additional set of 27 mesocosms was specifically set up for sampling at 30 DAS, although these were managed only under CF since water management up to 30 DAS was the same for both CF and AWD (see water management details below).

3.2.3. Experimental conditions

Water management: Soil moisture during straw incubation in the soil was maintained at 50% of field capacity (equivalent to a water content of 13% on a dry weight basis) gravimetrically. Just before flooding, pre-seeding fertilization was performed. Then all mesocosms were flooded, and water seeding was performed. Flooding was maintained for 30 days after seeding (DAS) by daily replacing the water lost by evapotranspiration. At 30 DAS (corresponding to tillering stage), the mesocosms were drained and fertilized. Half of the mesocosms (CF management) were re-flooded until the end of the experiment at 60 DAS. The other half were subjected to three 10-day AWD cycles each involving 4–5 days during which the water level in the bucket was gradually lowered to simulate the natural infiltration rates in the field, followed by 5 days of free drainage during which the soil was allowed to reach 73% water-filled pore space equivalent to a soil water potential of − 20 kPa.

Straw management: Natural abundance and ^{15}N-enriched rice straw were chopped into 0.5-cm segments and added to the soil 30 or 60 days before seeding. A straw application dose of 4.4 and 4.7 g kg^{-1} of soil d.w., equivalent to a field application dose of 10 Mg ha^{-1} d.w., was used for non-enriched and ^{15}N-enriched straw, respectively, in order to supply the same absolute amount of straw-derived N (26.7 mg N per mesocosm) due to small differences in their N contents.

Fertilizer management: Mineral N was added to the mesocosms in the form of ammonium sulphate just before seeding and at tillering stage (30 DAS). Both ^{15}N-enriched fertilizer (2 atom% ^{15}N) and natural abundance were used. The fertilizer was added to each mesocosm in solution by dissolving an appropriate amount of salt in 50 ml of deionized water. In addition, all mesocosms received a basal fertilization of potassium and phosphorus before seeding in the form of monopotassium phosphate (KH_2PO_4) and potassium chloride (KCl) at a dose corresponding to 15 kg P ha^{-1} and 50 kg K ha^{-1}.

Rice variety and growth conditions: One plant of *Oryza sativa* L. variety CL26 was established in each mesocosm. The seeds were pre-germinated on cotton before transferring to the soil mesocosms. The plants were grown in a controlled-environment growth chamber, equipped with LED lamps (Valoya, mod. LEDBX120C2), with 12 h of light (700 µmol m^{-2} s^{-1}) and 12 h of dark, at 20 °C.

3.2.4. Porewater analyses

Soil solution was collected by means of Rhizon samplers (Rhizon MOM 19.21.22, Rhizosphere, Wageningen, the Netherlands) installed vertically in proximity of the root system at a depth between 5 and 10 cm, with three replicates per treatments (only in SN mesocosms). Porewater sampling was performed approximately every 10 days from seeding (0, 12, 22, 36, 47, 57 DAS) and immediately analysed for reduced iron (Fe^{II}), ammonium (NH_4^+), nitrate (NO_3^-) and dissolved organic C (DOC) concentrations. Dissolved Fe^{II} was determined following the method descripted by Loeppert and Inskeep (1996) involving reaction with 1,10-phenanthroline under acid condition. Ammonium (NH_4^+) concentrations were determined spectrophotometrically by a modified Berthelot method involving reaction with salicylate in the presence of alkaline sodium dichloroisocyanurate (Crooke and Simpson, 1971). Nitrate (NO_3^-) concentrations in porewater samples were determined following the method descripted by Mulvaney (1996) which consists of quantitatively reducing NO_3^- to NO_2^- by addition of VCl_3 in the presence of Griess reagents and heating at 40 °C for 3 h. Dissolved organic C (DOC) was determined in acidified (pH = 2) aliquots of soil porewater by Pt-catalysed, hightemperature combustion (850 °C) followed by infrared and electrochemical detection of CO_2 and NO, respectively (Vario TOC, Elementar, Hanau, Germany) in a CO_2-free modified air carrier gas.

3.2.5. Soil analyses

Soil samples were collected from each mesocosm by means of a 10-mm diameter sampling probe at 30 and 60 DAS along the whole depth of the mesocosm explored by roots. Samples were immediately analysed for moisture content, inorganic N (ammonium and nitrate) content and microbial biomass C (MBC) as follows. Inorganic N was extracted from fresh soil samples with ammonium-free (NH_4^+ < 0.001%) 1 M KCl (soil:solution ratio 1:5). After shaking for 60 min at 80 rpm, samples were centrifuged at 800 × *g* for 10 min and the supernatant filtered through a membrane with a pore size of 2.5 μm (Whatman No. 42). Inorganic N in the extracts was determined spectrophotometrically as described above. Soil moisture content was also determined gravimetrically after drying an aliquot at 105 °C for 24 h, in order to express all concentrations on a dry soil weight basis.

The microbial biomass C (MBC) in the soil was determined by using the chloroform fumigation-direct extraction method (Murage and Voroney, 2007; Makarov et al., 2015; Setia et al., 2012). Soils were divided in two aliquots (10 g), one of which (non-treated) was extracted with 40 ml of 0.05 M K_2SO_4, while the other ($CHCl_3$-treated samples) was extracted with 40 ml of 0.05 M K_2SO_4 and 1 ml of ethanol-free chloroform. All samples were shaken for 1 h at 80 rpm, allowed to settle for 1 h and the supernatants decanted into clean containers that were subsequently ultracentrifuged for 15 min at 13,500 × *g* to obtain particle-free salt extracts. The chloroform-treated supernatants were purged with N_2 for 30 min to remove any residual $CHCl_3$. Total C concentrations in the extracts of the fumigated and non-fumigated samples were determined by a Vario TOC analyser (VarioTOC, Elementar, Hanau, Germany) using a combustion temperature of 680 °C. MBC was calculated by dividing the difference in DOC between the fumigated and non-fumigated extracts by an extraction efficiency value of 0.45 (Jenkinson et al., 2004).

3.2.6. Plant analyses

After soil sampling, plants were harvested at 30 DAS and 60 DAS at tillering and panicle differentiation stages, respectively. Roots were carefully washed with deionized water and then separated from shoots. Roots and shoots were dried at + 45 °C to determine the dry biomass. All vegetal materials were ground to a fine powder with an ultracentrifugal mill (Retsch mod. ZM 200). Total N contents and stable ^{15}N isotope composition of plant samples were determined with an automated elemental analyser continuous flow isotope ratio mass spectrometer (IRMS; Vario Isotope Select and IsoPrime100, Elementar, UK). Plant N uptake over the rice growth period was calculated as the product of shoot or root biomass and their N contents. The fraction of plant N derived from fertilizer (f_{FDN}) was calculated from ^{15}N isotope values by applying the following expression:

$$f_{FDN} = \frac{(at\%^{15}N_{15N\text{-}plant} - at\%^{15}N_{14N\text{-}plant})}{(at\%^{15}N_{15N\text{-}fertilizer} - at\%^{15}N_{14N\text{-}fertilizer})}$$

where at%^{15}N$_{15N\text{-plant}}$ and at%^{15}N$_{14N\text{-plant}}$ are the isotope ratio of shoot or root samples obtained from plants receiving enriched or natural abundance N fertilizer (^{15}N-fertilizer or ^{14}N-fertilizer), respectively (i.e. S15N and SN series). Natural abundance of ^{14}N fertilizer used was equal to 0.366 atom% ^{15}N. Fertilizer-derived N (FDN) in the shoots and roots was subsequently calculated as the product of f_{FDN} and N content in the respective plant part (N shoot or N root) and expressed in mg N mesocosm^{-1}. The fraction of plant N derived from the straw (f_{StDN}) was calculated in a similar way by applying a mixing model:

$$f_{StDN}=\frac{(at\%^{15}N_{15N\text{-plant}} - at\%^{15}N_{14N\text{-plant}})}{(at\%^{15}N_{15N\text{-straw}} - at\%^{15}N_{14N\text{-straw}})}$$

where at%^{15}N$_{15N\text{-plant}}$ and at%^{15}N$_{14N\text{-plant}}$ are the isotope ratio of shoot or root samples obtained from plants receiving enriched or natural abundance N straw (^{15}N-straw or ^{14}N-straw), respectively (i.e. 15SN and SN series). Natural abundance of ^{14}N straw used was equal to 0.368 atom% ^{15}N. Straw-derived N (StDN) in the shoots and roots was subsequently calculated as the product of f_{StDN} and N content in the respective plant part (N shoot or N root) and expressed in mg N mesocosm^{-1}. Soil-derived N (SDN) was determined as the difference between total plant N and the sum of fertilizer and straw-derived N. In order to obtain an agronomic index about the efficiency of fertilization under the different treatments and to better investigate the ability of plants to acquire fertilizer-N, the fertilizer-N use efficiency (FUE) was calculated as the ratio between FDN in total plant (obtained from the sum of FDN in shoot and FDN in root) and the amount of N applied with fertilizer. For the calculation of FUE at 30 DAS, only the amount of N applied in pre-seeding was considered.

3.2.7 Statistical analysis

Data from samples collected at 30 DAS and 60 DAS were treated separately because at 30 DAS, only the effects of straw and fertilization management were evaluated, while at 60 DAS, also the effects of water management were evaluated. Data collected at the end of the experiment (NH_4^+ and NO_3^- content in soil, MBC, N plant uptake, FDN, StDN, SDN, FUE) were tested by ANOVA. In particular, oneway ANOVA was applied for data at 30 DAS and two-way ANOVA for data at 60 DAS. The Shapiro–Wilk test and Levene's test were conducted to check the assumptions of normality and homogeneity of variance. When one of the assumptions was violated, a logarithmic transformation of data was applied. Treatment averages were separated by means of Bonferroni post hoc test at $p < 0.05$. Porewater data was analysed by applying the linear mixed-effect model for repeated measures with the "lme" function from the "nlme" R package. Statistical analysis was performed using R software version 4.0.5.

3.3. Results

3.3.1. Porewater and soil analyses

Changes in the concentrations of Fe^{II} in porewaters (Fig. 3.2) perfectly reflected water management. As expected, the Fe^{II} concentrations in CF generally increased after flooding and decreased with drainage at 30 DAS but maintained relatively high throughout the entire growing period. A similar increase in porewater Fe^{II} concentrations was observed in the first 30 DAS in AWD; however, with the start of the wetting and drying cycles after 30 DAS, soil oxidation resulted in a drop in porewater

Fe^{II} concentrations. Anticipated straw incorporation (S60) resulted in significantly lower porewater Fe^{II} concentrations with respect to straw incorporation near seeding (S30) in the 30 days after flooding, while no influence of fertilizer management was observed.

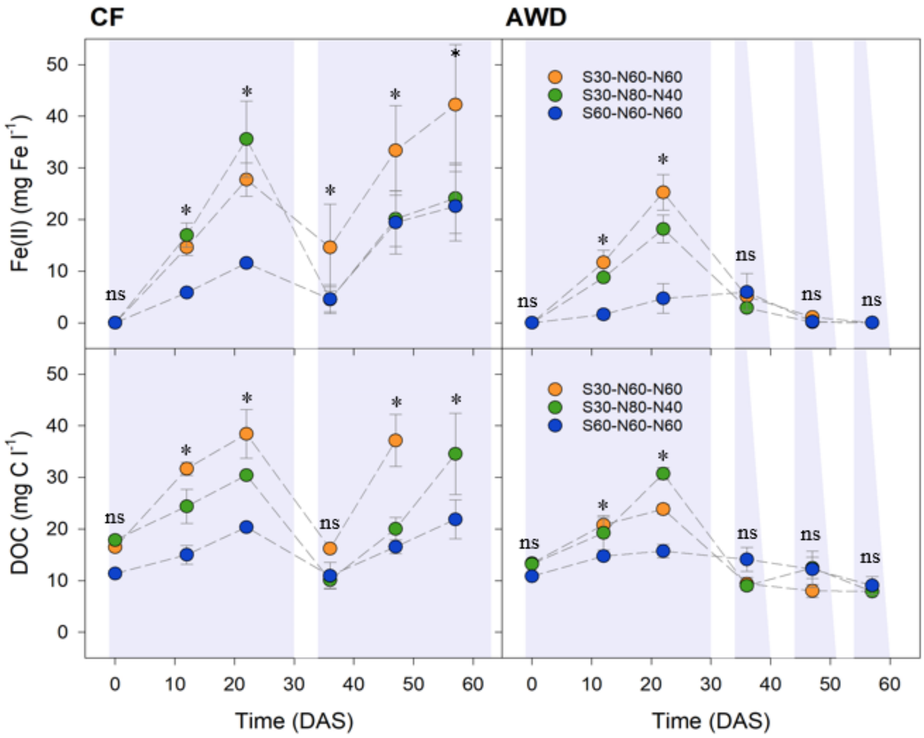

Figure 3.2 Variations in the concentration of reduced iron (Fe^{II}) and DOC in porewaters with time for continuous flooding (CF) and alternate wetting and drying (AWD) water management. Treatments involve straw incorporation 30 or 60 days before seeding (S30 and S60, respectively) and the splitting of applied N fertilizer between pre-seeding and tillering stage (60 + 60 vs 80 + 40 kg N ha^{-1}; N60-N60 and N80-N40, respectively). Error bars represent standard errors calculated on three replicates. The asterisk denotes a significant difference ($p < 0.05$) among treatments. Shaded areas represent the moments in which the mesocosms were water saturated.

Anaerobic conditions prevailing during the whole growth period in CF and in the first 30 DAS in AWD resulted in a general increase in DOC concentrations (Fig. 3.2) with time. In contrast, the drainage of mesocosms at tillering stage in both CF and AWD treatments and the consecutive wetting and drying between 30 and 60 DAS in AWD resulted in lower DOC concentrations. Both S30 treatments induced significantly higher concentrations DOC with respect to S60 during the first 30 DAS, while no effects of fertilizer-N management on DOC concentrations were observed.

Porewater NH_4^+ concentrations remained relatively low after the first N fertilization at sowing across all treatments and only showed a slight increasing trend over the first 30 days (Fig. 3.3), whereas exchangeable NH_4^+ contents were highest at 30 DAS (Table 3.2). During the early stages of plant development, available N was not significantly affected by the amount of fertilizer-N applied in pre-seeding as both S30-N60-N60 and S30-N80-N40 showed similar amounts of exchangeable NH_4^+ (Table 3.2). In contrast, anticipating the incorporation of crop residues (60 days before seeding in the S60-N60-N60) resulted in significantly lower available NH_4^+ contents with respect to the other treatments.

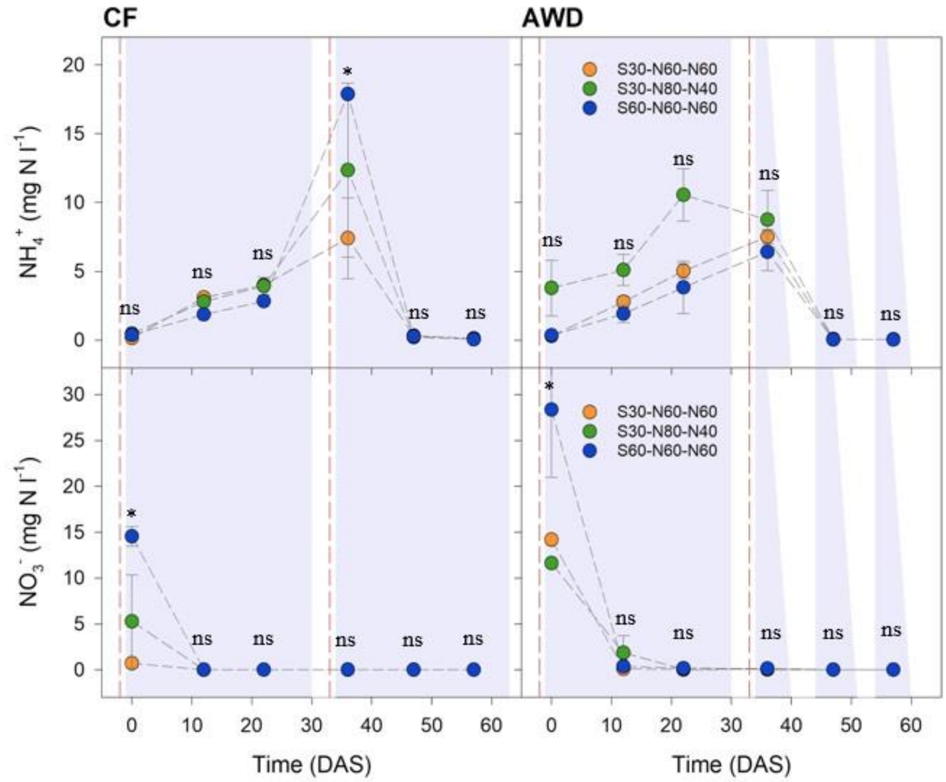

Figure 3.3 Variations in the concentration of ammonium (NH_4^+) and nitrate (NO_3^-) in porewaters with time for continuous flooding (CF) and alternate wetting and drying (AWD) water management. Treatments involve straw incorporation 30 or 60 days before seeding (S30 and S60, respectively) and the splitting of applied N fertilizer between pre-seeding and tillering stage (60 + 60 vs 80 + 40 kg N ha^{-1}; N60-N60 and N80-N40, respectively). Error bars represent standard errors calculated on three replicates. The asterisk denotes a significant difference ($p < 0.05$) among treatments. Shaded areas represent the moments in which the mesocosms were water saturated, while dashed red lines represent fertilizer-N applications.

Highest porewater NH_4^+ was observed immediately after fertilization at tillering stage (36 DAS), but porewater NH_4^+ concentrations subsequently decreased rapidly below detection limits in all treatments, probably due to the rapid plant N uptake and N losses. Similarly, exchangeable NH_4^+ contents in the soils were lower at 60 DAS than 30 DAS across all treatments, although contents were significantly lower under AWD with respect to CF (Table 3.2). A higher soil NO_3^- content under AWD than CF at 60 DAS was observed (Table 3.2). Nonetheless, porewater nitrate concentrations were generally below detection limits (Fig. 3), not only under the prevalent anoxic conditions when mesocosms were flooded (i.e. first 30 days in AWD and throughout the growth period in CF) but also during AWD cycles after tillering. High values were only recorded at seeding for the S60-N60-N60 treatment attributable to a greater degradation of early incubated rice straw, with a consequent net ammonium release and nitrification under oxidizing conditions.

Microbial biomass C in the soils did not show any significant differences between treatments irrespective of water, straw or fertilization management (Table 3.2).

Table 3.2 Ammonium (NH_4^+), nitrate (NO_3^-) and microbial biomass C (MBC) in soil at different sampling times (30 and 60 DAS) in different water management (continuous flooding (CF); alternate wetting and drying (AWD)) and straw and fertilizer treatments (S30-N60-N60, S30-N80-N40, S60-N60-N60). Data are given as mean values of the nine replicates ± standard deviation. Superscript lowercase letters indicate statistically significant differences between the treatments within water management ($p < 0.05$), while capital letters indicate statistically significant differences between water management ($p < 0.05$).

Sampling time	Water management[a]	Treatment	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})	MBC (mg C kg^{-1})
30 DAS	CF	S30-N60-N60	6.82 ± 2.34^{a}	0.16 ± 0.11	115.67 ± 107
		S30-N80-N40	6.31 ± 1.94^{ab}	0.10 ± 0.03	76.33 ± 29
		S60-N60-N60	4.22 ± 1.58^{b}	0.21 ± 0.23	117.5 ± 56
		Average	5.78	0.16	103.17
P(F) Treatment			0.024	ns	ns
60 DAS	CF	S30-N60-N60	2.75 ± 1.77	0.19 ± 0.06	149.12 ± 172
		S30-N80-N40	2.44 ± 1.12	0.23 ± 0.08	105.2 ± 78
		S60-N60-N60	2.20 ± 0.88	0.23 ± 0.03	124 ± 146
		Average	2.46 A	0.22 B	126.11
	AWD	S30-N60-N60	0.45 ± 0.38	0.27 ± 0.04	136.88 ± 61
		S30-N80-N40	0.21 ± 0.23	0.34 ± 0.10	142.3 ± 41
		S60-N60-N60	0.25 ± 0.26	0.33 ± 0.06	109.67 ± 49
		Average	0.30 B	0.31 A	129.63
P(F) Water management			ns	0.020	ns
P(F) Treatment			0.000	0.000	ns
P(F) Water management*Treatment			ns	ns	ns

[a] CF: continuous flooding; AWD: alternate wetting and drying

3.3.2. Plant analyses

The adoption of AWD generally resulted in a lower plant development over the studied growth period, related to significantly lower leaf and especially root development, resulting in a higher, although not significant, shoot-to-root ratio than CF (Table 3.3). As expected, a higher pre-seeding N fertilization in the S30-N80-N40 treatment determined a significantly higher shoot and root biomass at 30 DAS with respect to the other treatments that received 25% less fertilizer-N. This difference was still observed at 60 DAS only under CF whereby plants receiving a higher amount of mineral N in pre-seeding (i.e. S30-N80-N40 treatment) had significantly higher biomass that those receiving a more balanced splitting of the fertilizer-N between pre-seeding and tillering (i.e. S30-N60-N60 treatment; Table 3.3). Water management*treatment interaction showed that the incorporation of rice straw close to seeding in CF resulted in a significantly higher plant growth, compared to the early straw incorporation in CF, while under AWD, plant growth was similar across all treatments (Table 3.3).

AWD generally reduced total plant N uptake by about 4–25% in comparison to CF depending on the treatment (Fig. 3.4). Similar to what was observed for total plant biomass, a higher pre-seeding fertilizer-N dose led to a significantly higher plant N uptake at 30 DAS. On the other hand, in both CF and AWD, plant N uptake at 60 DAS was mainly influenced by the timing of straw addition with an early straw incorporation (S60) resulting in a significantly lower total plant N uptake with respect to a late incorporation (S30). Under CF irrigation, the best treatment for rice N nutrition appeared to be the S30-N80-N40, while in AWD, the S30-N60-N60 showed the highest total plant N uptake at 60 DAS. The effects of water management and straw and fertilizer treatments on total shoot and root N mirrored what was observed for total plant N uptake suggesting no treatment effect on the distribution of plant N between different parts of the plant.

*Table 3.3. Shoot and root dry biomass and shoot-to-root ratio of plants at different sampling times (30 and 60 DAS) for the different water managements (continuous flooding (CF); alternate wetting and drying (AWD)) and straw and fertilizer treatments (S30-N60-N60, S30-N80-N40, S60-N60-N60). Data are given as mean values of the nine replicates ± standard deviation. Superscript letters indicate statistically significant differences between treatments at 30 DAS; superscript italic letters indicate statistically significant differences for water management*treatment interaction at 60 DAS ($p < 0.05$), while capital letters indicate statistically significant differences between water managements ($p < 0.05$).*

Sampling time	Water management[a]	Treatment	Shoot dry biomass (g mesocosm^{-1})	Root dry biomass (g mesocosm^{-1})	Shoot-to root ratio
30 DAS	CF	S30-N60-N60	0.58 ± 0.06^{b}	0.35 ± 0.07^{ab}	1.67 ± 0.28
		S30-N80-N40	0.76 ± 0.15^{a}	0.43 ± 0.09^{a}	1.77 ± 0.23
		S60-N60-N60	0.56 ± 0.07^{b}	0.32 ± 0.05^{b}	1.80 ± 0.39
		Average	0.63	0.37	1.75
P(F) Treatment			0.004	0.006	ns
60 DAS	CF	S30-N60-N60	4.44 ± 0.38^{b}	3.29 ± 0.43^{b}	1.36 ± 0.12
		S30-N80-N40	5.06 ± 0.31^{a}	4.08 ± 0.68^{a}	1.26 ± 0.17
		S60-N60-N60	3.75 ± 0.45^{c}	2.51 ± 0.31^{c}	1.51 ± 0.22
		Average	4.41 A	3.30 A	1.38
	AWD	S30-N60-N60	4.13 ± 0.50^{bc}	2.66 ± 0.43^{c}	1.57 ± 0.15
		S30-N80-N40	3.66 ± 0.47^{c}	2.22 ± 0.47^{c}	1.72 ± 0.41
		S60-N60-N60	3.56 ± 0.45^{c}	2.12 ± 0.21^{c}	1.68 ± 0.19
		Average	3.78 B	2.33 B	1.66
P(F) Water management			0.000	0.000	ns
P(F) Treatment			0.000	0.000	0.000
P(F) Water management*Treatment			0.000	0.000	ns

[a] CF: continuous flooding; AWD: alternate wetting and drying

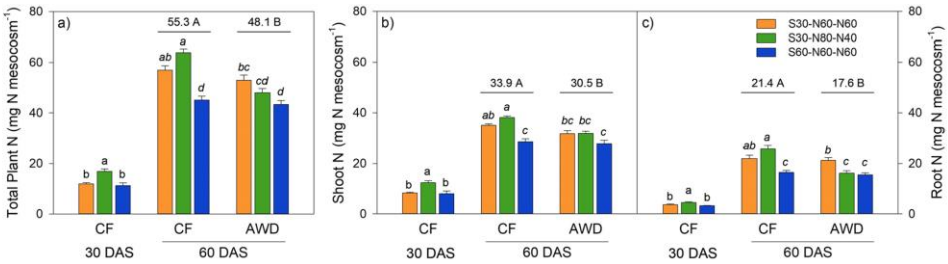

*Fig. 3.4 Total plant N content (a) and contribution of shoot (b) and root (c) N to total plant N as a function of water management (continuous flooding (CF); alternate wetting and drying (AWD)) and straw and fertilizer treatments (S30-N60-N60, S30-N80-N40, S60-N60-N60). The numbers at the top show the average plant N for each water management at 60 DAS with capital letters indicating statistically significant differences between averages ($p < 0.05$). Lowercase letters indicate statistically significant differences between the treatments at 30 DAS ($p < 0.05$), while lowercase italic letters indicate statistically significant difference for interaction water management*treatment at 60 DAS ($p < 0.05$)*

Figure 3.5 shows the relative contribution of fertilizer-, straw- and soil-derived N to rice N uptake in the shoots and roots as affected by water, straw and fertilizer management. This source partitioning of N allowed to observe that fertilizer-derived N (FDN) and soil-derived N (SDN) where the main contributors to total plant N, with straw-derived N generally contributing less than 5% of total plant N, across treatments. The contribution of FDN to both shoot and root N was significantly higher in CF than AWD (Fig. 3.5a, b), with early incorporation of rice straw (i.e. S60-N60-N60) leading to the highest amount of FDN especially in the shoot under both CF and AWD at 60 DAS. SDN, the other major contributor of N to the plant, was significantly higher in plants grown under CF than AWD (Fig. 5c, d). Treatments with straw incorporation 30 days before sowing (i.e. S30-N80-N40 and S30-N60-N60) showed a significantly higher contribution of SDN to plant uptake with respect to the treatment where straw was incorporated earlier (i.e. S60-N60-N60) under both CF and AWD water management. Water management did not affect the contribution of rice straw-derived N (StDN) to shoot N, although a significantly higher StDN in the roots was observed under CF with respect to AWD (Fig. 5e, f). A maximum percentage of 2.3% of shoot N and 3.4% of root N was derived from incorporated straw in the S30-N60-N60 treatment under CF at 60 DAS. As for SDN, rice straw management was the main driver of the main differences in StDN observed between the treatments. In fact, in most cases, the incorporation of rice straw 30 days before seeding resulted in a greater contribution of StDN to plant N with respect to an early incorporation 60 days before seeding.

Considering the total amount of plant N derived from the applied fertilizer-N, CF showed a significantly higher mean fertilizer use efficiency (FUE) with respect to AWD at 60 DAS (Fig. 3.6). However, FUE under CF was not substantially influenced by the different straw and fertilizer treatments; the timing of straw incorporation strongly affected FUE under AWD. In fact, whereas a similar FUE was observed under both water managements when rice residues were incorporated 60 days before seeding, their late incorporation (in S30-N60-N60 and S30-N80-N40) resulted in significantly lower efficiencies under AWD.

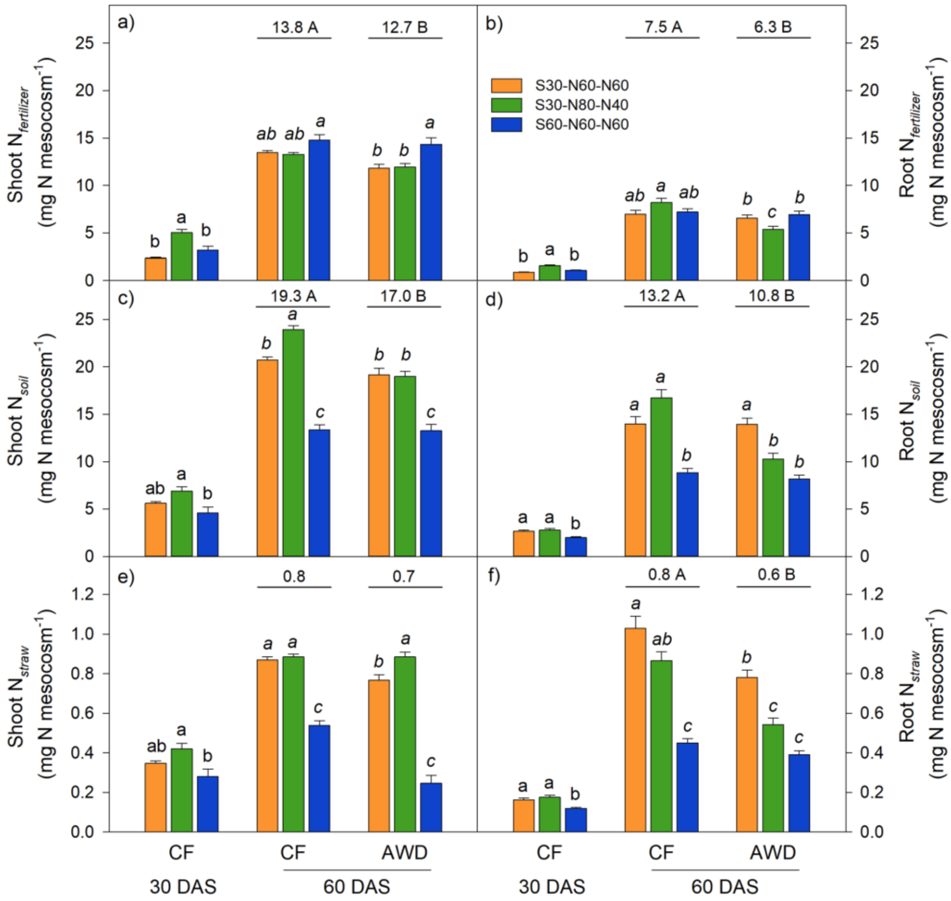

*Fig. 3.5 Contribution of fertilizer (a, b), soil (c, d) and straw (e, f) to plant N uptake in the shoot (a, c, e) and root (b, d, f) as a function of water management (continuous flooding (CF); alternate wetting and drying (AWD)) and straw and fertilizer treatments (S30-N60-N60, S30-N80-N40, S60-N60-N60). The numbers at the top show the average shoot or root N for each water management at 60 DAS with capital letters indicating statistically significant differences between averages ($p < 0.05$). Lowercase letters indicate statistically significant differences between the treatments at 30 DAS ($p < 0.05$), while lowercase italic letters indicate statistically significant difference for interaction water management*treatment at 60 DAS ($p < 0.05$).*

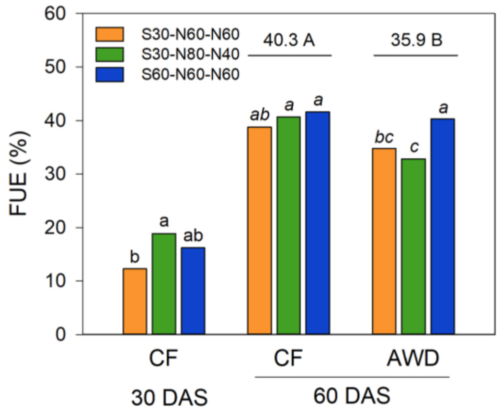

*Fig. 3.6 Fertilizer use efficiency (FUE) as a function of water management (continuous flooding (CF); alternate wetting and drying (AWD)) and straw and fertilizer treatments (S30-N60-N60, S30-N80-N40, S60-N60-N60). The numbers at the top show the average FUE for each water management at 60 DAS with capital letters indicating statistically significant differences between averages ($p < 0.05$). Lowercase letters indicate statistically significant differences between the treatments at 30 DAS ($p < 0.05$), while lowercase italic letters indicate statistically significant difference for interaction water management*treatment at 60 DAS ($p < 0.05$).*

3.4. Discussion

3.4.1. Influence of water management on N availability and plant uptake

With respect to CF, the adoption of AWD cycles after tillering was shown to result in a lower plant growth during the early vegetative stages, particularly for root development, in line with previous findings (Suriyagoda et al., 2014; Weerarathne et al., 2015; Zhou et al., 2020; Wu et al., 2022). Several authors have however observed a positive plant growth response under AWD, with an increase in root length and dry matter accumulation (Kato and Okami, 2010; Thakur et al., 2011; Hazra and Chandra, 2016; Abid et al., 2022). It is well-known that rice plants grown under AWD can regulate the growth of above and belowground biomass in different proportions depending on the stage of the growing cycle (Zhang et al., 2021). Therefore, the lower root-to-shoot ratio under AWD at panicle initiation stage observed in this experiment could be compensated at later phenological stages. This was already shown by Somaweera et al. (2016), among other studies, where the relationship between AWD and plant growth at field scale was investigated taking into consideration the entire crop cycle until harvest. Moreover, in our mesocosm experiment, the limited volume of soil and different environmental conditions may have differently affected soil physicochemical properties and crop growth compared to field conditions (Jin et al., 2020).

The lower N uptake under AWD can be probably attributed to a lower root development, as previously observed by Barison and Uphoff (2011), as well as the well documented extensive N losses that occur during the consecutive redox cycles of AWD (Miniotti et al., 2016; Zhang et al., 2018). Indeed, it is reasonable to attribute the lower soil NH_4^+ availability observed under AWD irrigation to greater nitrification–denitrification losses typical of AWD management. Leaching losses during AWD cycles were deemed negligible as the analysis of drainage waters from the tubes showed nitrate concentrations that where below detection limits (data not shown).

Water management did not only affect the total plant N uptake but also the source partitioning of the assimilated N. Under both water managements, SDN and FDN contributed most to plant N at 60 DAS (46–64 and 34–49%, respectively), while StDN only contributed as a minimal fraction (3% for both CF and AWD), in line with the findings of Wu et al. (2022) who also reported a similar partitioning of rice N uptake under AWD. Our results agree with those reported by Chen et al. (2016)

who reported that 34–42% of rice plant N was derived from fertilizer-N and 58–66% from soil N. Similarly, Hashim et al. (2015) reported FDN values for rice ranging from 20 to 35%.

Although rice plants displayed a lower N content under AWD irrigation, the FDN and consequently fertilizer use efficiency were only slightly affected by water management (Figs. 3.5 and 3.6) suggesting that the observed differences in N nutrition between different irrigations regimes were not exclusively due to higher fertilizer-N losses under AWD. In fact, Cucu et al. (2014) observed that the increased retention of fertilizer-N in flooded with respect to non-flooded soils could actually contribute to limit N losses. In light of this, we speculate that fertilizer-N immobilization during the first 30 days of flooding could have partly limited or delayed N losses during the successive AWD redox cycles after tillering. Zhu et al. (2022) actually report a higher fertilizer-N recovery under AWD than CF, suggesting that the slightly lower FUE observed during the early growth stages in our experiment under AWD (35.9%) compared with CF (40.3%) can probably be recovered during the later growth stages allowing to reach similar or higher values of N uptake at harvest (Yang et al., 2004; Wang et al., 2016).

Unlike FDN, the fraction of SDN was greatly affected by water management, showing a significantly lower contribution to plant N nutrition under AWD (on average 14% less) with respect to permanent flooding irrigation (Fig. 3.5), in line with the findings of Wu et al. (2022). This is in contrast with the faster mineralization of soil organic N during the more frequent oxic soil conditions we hypothesized for AWD (Hypothesis 1). Previous studies have shown that, under predominantly anoxic conditions, Fe-reducing bacteria may use Fe^{III} in Fe oxides as an electron acceptor leading to the reductive dissolution of these Fe minerals that are also known to stabilize important amounts of organic matter. This consequently leads to the release of Fe^{II} together with substantial amounts of dissolved organic matter into solution (Said-Pullicino et al., 2014) that may serve as an important pool of labile organic N and, through mineralization, as a source of SDN for plant uptake (Deroo et al., 2021). On the other hand, the periodic fluctuations in redox conditions during AWD cycles can promote the co-precipitation of dissolved organic matter (Sodano et al., 2017), potentially enhancing its stabilization against microbial decomposition and reducing the contribution of SDN to plant nutrition. In fact, our results evidenced that whereas Fe^{II} and DOC porewater concentrations rapidly increased when soils were flooded during the first 30 DAS, the concentrations where substantially lower with the onset of AWD cycling at tillering with respect to CF (Fig. 3.2).

Water management only slightly influenced the contribution of StDN to plant N, nonetheless showing a lower contribution under AWD with respect to CF (Fig. 3.5), as previously reported by Zhang et al. (2021). Although we expected AWD cycles to promote microbial activity responsible for rice straw decomposition (Hypothesis 1), the different redox conditions between the irrigation regimes could have resulted in a shift in microbial communities with different N demands (Reddy et al., 1986). In contrast to anaerobic microorganisms, aerobic microorganisms are known to have high metabolic N requirements (Gale et al., 1992; Reddy and deLaune, 2008), and therefore, the faster degradation of labile organic substrates like straw under AWD (Borken and Matzner, 2009) could nonetheless be associated with a slower release of StDN for plant uptake due to the enhanced microbial immobilization of N released from labile sources (Ponnamperuma, 1972).

3.4.2. Influence of straw management on N availability and plant uptake

The timing of rice straw incorporation with respect to seeding and soil flooding was the primary driver controlling plant N nutrition during the early vegetative stages in fertilized paddy soils under both water managements and strongly influenced the source partitioning of N assimilated by the plant. Changing the time period between crop residue incorporation and the beginning of the cropping season can influence both the balance between residue N mineralization and immobilization before cropping and consequently also the availability of inorganic N forms and labile organic substrates at the time of flooding, with important implications on the availability of FDN, SDN and StDN. Indeed, we hypothesized that the anticipation of residue incorporation allows more time for the aerobic decomposition of incorporated straw before soil flooding (and the shift from a net N immobilization to a net N mineralization phase), thus enhancing the availability of StDN for plant uptake and limiting the immobilization of applied fertilizer-N during the early stages of plant development (Hypothesis 2). Results however evidenced that an early incorporation of rice straw (i.e. 60 days before seeding) substantially decreased total plant N uptake, particularly under CF (Fig. 3.4), suggesting that this practice did not bring the expected benefit to plant growth and nutrition. In contrast, rice straw incorporation near seeding favoured plant N uptake, especially under CF. Source partitioning of plant N evidenced that the relative contribution of both straw- and soil-derived N was lower with early incorporation, while FDN was slightly higher but not sufficient to compensate for the lower contribution from other N sources (Fig. 3.5). In fact, the higher FDN and FUE with early straw incorporation were expected (Hypothesis 2), because it avoids the high availability of labile organic matter in correspondence with mineral N fertilization when residues (with a C/N ratio of around 62.5) are incorporated close to seeding and therefore limits the microbial immobilization FDN to the benefit of plant uptake (Said-Pullicino et al., 2014). On the other hand, promoting straw decomposition and organic N mineralization under aerobic conditions with early incorporation negatively affected the contribution of StDN to plant uptake, falsifying our second hypothesis. This was probably because most of the plant-available StDN released before seeding was nitrified and rapidly lost by denitrification with the onset of soil flooding (Mikkelsen, 1987), as well as during the successive AWD cycles. This was confirmed by the higher porewater NO_3^- concentrations at seeding (i.e. 0 DAS in Fig. 3) that were however immediately lost within 12 days from flooding.

The strongest effect on the timing of straw incorporation was however observed on the contribution of SDN to plant nutrition. Here, the incorporation of crop residues in proximity of flooding positively affected the supply of indigenous N, particularly under CF. We explained this by considering the positive feedback straw-derived C could have on SDN availability under anaerobic conditions. Indeed, under these conditions, freshly incorporated residues serve as a source of labile substrates for the C-limited Fe-reducing bacteria, thereby promoting the reductive dissolution of Fe oxyhydroxides and release of associated organic matter into the soil solution (Marschner, 2021). This was confirmed by the increasing trend and higher porewater DOC and Fe^{II} concentrations observed during the early days of plant development in soils receiving straw 30 days before flooding (Fig. 3.2). Desorbed (and therefore destabilized) soil organic matter can subsequently serve as an important source of indigenous N supply (Akter et al., 2018; Deroo et al., 2021), thereby improving the contribution of SDN to total plant uptake. On the other hand, anticipating the incorporation of rice straw enhances their degradation under aerobic conditions, thereby decreasing the amount of straw-derived labile C available to support microbial activity under anaerobic conditions with the onset of soil flooding (Wang et al., 2015), and consequently, the desorption of soil organic matter that can serve as a source of indigenous N supply is less pronounced. Similar effects of the timing of crop residue incorporation on the reductive dissolution of Fe oxyhydroxides and the release of Fe^{II} and soil-derived organic matter into solution were also reported in the field (Bertora et al., 2018b). The positive effect of rice straw incorporation on indigenous N supply was less expressed under AWD (Fig. 3.5) where the regular introduction of oxygen during the redox cycling limited Fe reduction DOC desorption after tillering (Fig. 3.2).

3.4.3. Influence of fertilizer splitting on N availability and plant uptake

Sustaining plant growth through an adequate nutrient supply greatly depends on the temporal synchrony between N supply and plant demand during the different stages of crop development. In this context, the timing of fertilizer-N application and the feedback on N supply from other sources have an important bearing on N availability for microbial activity and plant uptake alike. By evaluating the effects of different fertilizer-N splitting on the source-differentiated N uptake by rice plants, we showed that applying higher fertilizer-N doses before seeding in order to temporarily favour fertilizer incorporation in the microbial biomass and limit FDN losses during the AWD cycles was not effective to increase contribution of FDN to plant N uptake or FUE (Figs. 3.5 and 3.6) over the experimental period studied, thereby rebutting our third hypothesis. We cannot however exclude that the benefits of a high fertilization dose at seeding under AWD are eventually observed at the later stages of plant development and that the release of immobilized fertilizer-N can actually contribute to plant N uptake after 60 DAS (beyond our experimental period) due to the higher N demand of the aerobic microbial population (Somaweera et al., 2016). This aspect warrants further investigation. There was however a slight but significant positive effect on root development under flooded conditions, as previously observed by Yang et al. (2021).

Higher fertilization doses at seeding did however enhance the contribution of SDN, and to a much smaller extent StDN, to plant uptake at 30 DAS (Fig. 3.5). This actually led to a 17% increase in the contribution of SDN to total plant N uptake by 60 DAS under CF, but no significant difference was observed with respect to StDN (Fig. 3.5). We postulate that the greater root biomass observed for this N fertilizer split ratio (Table 3.3) could have also promoted belowground C allocation in the form of rhizodeposited C, thereby resulting in microbial activation and a positive rhizosphere priming effect on soil-derived organic matter mineralization, similar, but to a lesser extent, to what was observed with rice straw incorporation. Luo et al. (2019) have shown that N fertilization can increase the allocation of plant photosynthates into the rice rhizosphere as a result of a higher root biomass. In addition, Zhu et al. (2018) and Jiang et al. (2021) have shown a relationship between root C exudation, C (and N) availability for microbes and a positive rhizosphere priming at high N fertilization rates (as those used in this experiment). In fact, the combination of enhanced fertilizer-N uptake and increased root

C exudation by the plant may induce a strong increase in competition between plants and microorganisms for N inducing N limitation in the rhizosphere which induces microbes to accelerate the mineralization of SOM to obtain nutrients.

On the other hand, the relative contribution of SDN to total plant N under AWD was slightly but significantly higher when N fertilizer dose was split equally between pre-seeding and tillering suggesting that a more regular temporal distribution of fertilizer-N could favour microbial activity and indigenous N supply. These findings further suggest that the added-N interaction of N fertilization on soil-derived N uptake in rice paddies may not only depend on the fertilizer-N input rates (Sun and Zhu, 2022) but also on soil redox conditions.

3.5. Conclusions

The widespread adoption of AWD water management in rice paddies to improve the water use efficiency and environmental sustainability of rice cropping systems will depend on avoiding yield gaps with respect to the conventional continuous flooding practices. Providing adequate N supply for rice plants under AWD, particularly during the early vegetative stages, is one of the most pressing challenges. N supply from different sources depends on the complex interactions between water, fertilizer and crop residue management which need to be specifically optimized to enhance plant N uptake. In this study, we provide important insights into the influence of management practices on the source partitioning of plant N uptake. The main findings of this work can be summarized as follows:

1) Soil-derived N was the main source of N for rice plants at panicle initiation stage (46–64%), followed by fertilizer-derived N (34–49%), while straw-derived N only contributed minimally (< 3%). Despite

the low contribution of crop residues to plant nutrition, their incorporation can play a crucial role in enhancing soil N supply by promoting the positive feedback on soil organic matter desorption under anaerobic conditions that can in turn serve as an important pool of labile organic N and a source of SDN for plant uptake.

2) Although AWD reduced total N uptake by about 4–25% with respect to continuous flooding, this could only be partly attributed to higher fertilizer-N immobilization or losses as a result of redox cycling, suggesting that other N sources were affected by water management. In fact, the contribution of SDN to plant N uptake was strongly related to redox conditions, with a higher soil N supply observed under continuous flooding, particularly when straw was incorporated in proximity to flooding (61–64% of total plant N). Indeed, the combination of a fresh organic matter supply and reducing conditions under continuous flooding favoured the reductive dissolution of Fe oxyhydroxides and the desorption of soil organic matter that presumably increase soil N supply via mineralization.
3) Under continuous flooding, higher N fertilization doses at seeding may also enhance organic matter decomposition, thereby priming soil N supply, although the opposite was true under AWD probably due to the different metabolic N requirements of the microbial populations. From an agronomic point of view, an equilibrated splitting of N fertilizer between pre-seeding and tillering stages could favour microbial activity under AWD improving N supply from straw and soil organic matter degradation.

Although our study has highlighted how management practices may modulate the contribution of different N sources to plant nutrition, most of these effects are a result of changes in plant–microbe interactions in the rhizosphere that are not always unequivocal. This warrants further research to understand how these interactions are influenced by changes in soil redox conditions and their implications on plant nutrition, in order to provide useful indications for N management in rice paddies.

4. Conservation tillage in temperate rice cropping systems: Crop production and soil fertility

4.1. Introduction

Rice is the second most important cereal crop in the world with 194 Mha cultivated globally, and Italy stands out as the main rice producer in Europe, with an area of 227.320 ha (FAOSTAT, 2020). In Italy rice is cultivated once per year from the end of April until the beginning of October. Soil preparation commonly involves three or four operations, depending on the soil characteristics: mouldboard ploughing, which is carried out in either autumn or spring, followed in spring by laser leveling and one or two harrowing (Cordero et al., 2018; Miniotti et al., 2016). These conventional tillage practices provide high grain yields, but their sustainability in rice cropping systems has often been questioned primarily due to their negative effects on soil organic matter (SOM) mineralization, soil physical, chemical and biological fertility (Chen et al., 2007). In addition, conventional tillage leads to high costs due to higher energy demand and longer time required for seedbed preparation (Calcante and Oberti, 2019). Therefore, alternative soil management practices that allow to reduce agronomic, environmental, and economic impact of European temperate rice cultivation, while maintaining high yields, deserve to be investigated (Miniotti et al., 2016; Moreno-García et al., 2020).

Conservation agriculture can be a viable alternative to conventional management in rice cropping systems (Huang et al., 2015). Among the three pillars of conservation agriculture (reduced mechanical soil disturbance, permanent soil cover using crop residues or cover crops, and crop rotation), reduction of soil tillage intensity, i.e. minimum tillage and no tillage, is the one which is currently being adopted by a certain extent in Italian rice cropping systems, and its application is continuously increasing (Ferrero et al., 2021). Indeed, rice in Italy is mainly cultivated as monocrop and the use of cover crops is limited, even though their cultivation has increased over the last years, particularly in organic rice cultivation (Fogliatto et al., 2021; Vitalini et al., 2020).

The benefits of conservation tillage on rice crop yield generally depends on climatic conditions, soil type, cultivar and agronomic practices adopted (Huang et al., 2015). Conservation tillage in subtropical regions was shown to increase rice yield by 3.4% to 4.1% when compared to conventional tillage (Denardin et al., 2019; Zheng et al., 2014), though similar (Xu et al., 2010) or even decreased yields (Huang et al., 2015) have been previously reported. Moreover, information about the effects of conservation tillage on rice grain yield in temperate continuously flooded rice is still lacking, and the few studies available have reported 10–20% reductions in yields with no-tillage when compared to ploughing (Cordero et al., 2017; Perego et al., 2019).

The reduction of tillage in paddy soils generally results in increased soil bulk density in the surface layer and thus increased compaction (Kahlon, 2014), which is already favored by the typical flooded conditions of rice cultivation (Sacco et al., 2012). Therefore, seed germination, seedling establishment and root development can be hampered, eventually resulting in yield reduction (Busari et al., 2015; Munkholm et al., 2013; Tesfahunegn, 2015). For cereals other than flooded rice, the higher compaction under no-tillage can be mitigated after a few years of continuous adoption by the soil self-structuring capacity (Blanco-Canqui e Ruis, 2018). This can contribute to reduce yield losses compared with conventional tillage in the long term also in flooded rice cropping systems, thought depending on seeding techniques and climatic conditions (Jat et al., 2014; Zheng et al., 2014). It is well known how conservation tillage methods contribute to improve paddy soil quality and environmental sustainability, by favoring soil organic carbon (SOC) storage and reducing soil aggregate breakdown, even in the short term (Huang et al., 2012; Xue et al., 2015). However, in paddy soils managed with conservation tillage, the mulching effect of crop residues left on the soil surface results in lower soil temperatures, and together with increased soil compaction, delays N cycling (Bird et al., 2003; Li et al., 2015) and rice N uptake (Eagle et al., 2000). Indeed, SOM decomposition rates are lower under conservation than conventional tillage systems due to the physical protection of SOM within soil aggregates that reduces the exposure of labile SOM pools to degradation and mineralization by biological activity (Jin et al., 2011; Maltas et al., 2013).

Therefore, to compensate for the lower N availability due to the slower SOM mineralization and the consequent negative effects on rice yield under conservation tillage, these alternative techniques may require increased rates or a different splitting strategy in N fertilization compared to conventional tillage (Huang et al., 2018; Lundy at al, 2015).

Several authors demonstrated that in subtropical and tropical areas conservation tillage promotes SOC accumulation in paddy soils, particularly when these alternative tillage methods are applied in the medium to long term, i.e. more than 6 years (Carlos et al., 2022; Huang et al., 2012; Wang et al., 2019). The SOM stratification induced by the non-inversion of the soil layers with reduced tillage, results in higher SOM contents in the superficial soil layers that decrease progressively with soil depth (Varvel and Wilhelm, 2011). On the contrary, conventional systems determine a homogeneous SOM distribution in the topsoil because crop residues are incorporated to greater depths, that also favor SOM decomposition as a result of the breakdown of soil aggregates (Qi et al., 2021; Xue et al., 2015). This induces the formation of smaller aggregates, with low C content, and free particulate organic matter, characterized by less stability and faster turnover (Zhu et al., 2014).

Long and medium-term adoption of conservation tillage and its effects on rice yield and on SOC dynamics have already been studied in tropical and subtropical areas (Carlos et al., 2022; Huang et al., 2015; Wang et al., 2021). In temperate climates the effects of these techniques have been investigated in many cropping systems (Fiorini et al., 2020; Krauss et al., 2017; Van den Putte et al., 2010), however there is a lack of knowledge concerning the effects on paddy soils in medium-term applications.

Building upon these considerations, this work aims to evaluate the adoption of conservation tillage in the medium term for rice cultivation in temperate climate areas as an alternative to conventional tillage, and particularly evaluate whether conservation tillage can provide high grain yields by increasing soil fertility. We hypothesized that:

(1) **conservation tillage decreases grain yield, but in the medium-term stabilization of yield at levels comparable to conventional tillage can occur due to improved soil fertility;**

(2) **increasing mineral fertilization with N allows to fill the yield gap in conservation tillage compared with conventional tillage;**

(3) **conservation tillage increases SOC stocks even in temperate rice cropping systems, where the only OM input to the soil is crop residues, thus increasing the environmental sustainability of these cropping systems;**

(4) **conservation tillage accumulates labile and physically protected OM in the superficial layers of paddy soils, increasing N availability for the rice plant.**

To test these hypotheses, we compared conventional and conservation tillage in a medium-term field experiment evaluating their effects on rice yield and yield components, on soil bulk density and SOM fractions distribution in the soil profile.

4.2. Material and methods

4.2.1. Experimental site and pedoclimatic characteristics

A rice field experiment was carried out from 2014 to 2019 within a medium-term continuous rice monocrop experimental field. The site was located in the western part of the Po River valley (Pieve Albignola, NW Italy; 45◦06’41.2" N, 8◦57’06.2" E), representing the main Italian paddy area.

According to Köppen-Geiger (Köppen, 1936), climate in the area is defined as *Cfa*, with hot summers, cold winters and two main rainy periods in spring and autumn. Total yearly rainfall was highly variable during the experimental period (Fig. 4.1), ranging from 916 to 371 mm, but nevertheless lower than the mean total annual precipitation over the last 10 years (952 mm). Mean annual minimum and maximum temperatures were close to 0 and +25 °C, respectively, while the mean annual temperature (+13.6 °C) was slightly higher than the last decade (+12.9 °C).

According to the USDA soil taxonomy (Soil Survey Staff, 2010), the soil of the experimental field was an Ultic Haplustalf, sandy loam, mixed, mesic. The content of sand (2–0.05 mm), silt (0.05–0.002 mm) and clay (<0.002 mm) was corresponding to 63%, 30% and 7%, respectively. The topsoil (0–30 cm) was chemically characterized as follows: acidic pH (in H_2O), 5.7; medium soil total N content (Kjeldahl), 1.3 g kg^{-1}; high organic matter content (Walkley and Black), 19.0 g kg^{-1}; medium-high cation exchange capacity (ammonium acetate method, pH 7), CEC: 9.7 cmol+ kg^{-1}, where exchangeable Ca^{2+}, Mg^{2+} and K^{+} were 510.5, 63.9 and 72.7 mg kg^{-1}, respectively.

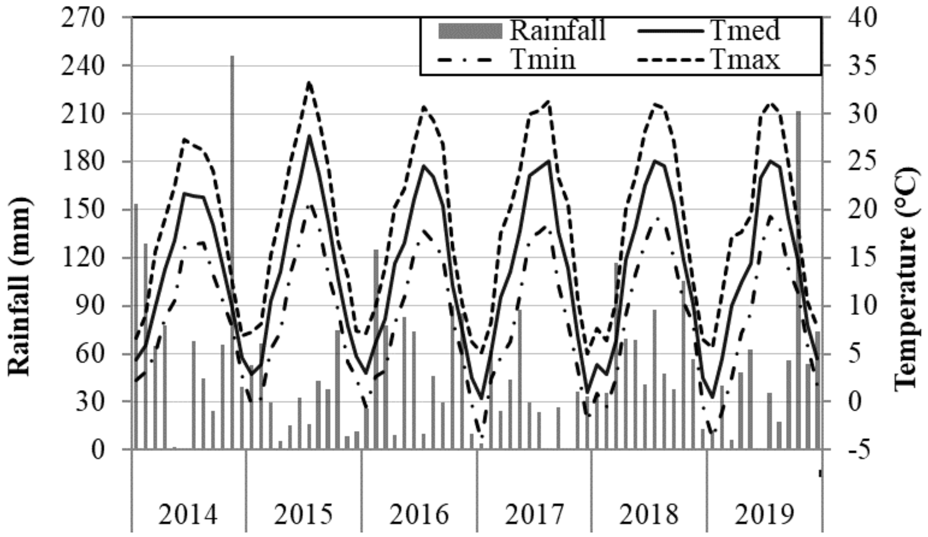

Fig. 4.1. Maximum, minimum and average monthly temperature and precipitation from 2014 to 2019.

4.2.2. Experimental setup and agronomic management

The experimental design was a split plot with two experimental factors: tillage practices in the main plots and N fertilization rates in the subplots. Three different tillage practices were compared for seedbed preparation:

(1) conventional tillage (*CT*), managed with reversible mouldboard plough with 30 cm working depth, followed by one disc harrowing and dry seeding; (2) minimum tillage (*MT*) managed with one passage of a combined cultivator (chisels and discs) with 10 cm working depth, followed by one disc harrowing and dry seeding; (3) no-tillage (*NT*) that implied a sod-seeding management performed with a sod-seeder with planter unit consisting of a single disk. The three tillage treatments were conducted in plots of about 600 m^2 and were set up in a randomized complete block design with three replicates. In both *CT* and *MT*, tillage was performed in spring and dry seeding was carried out using a pneumatic seed drill to uniform with *NT* management. After harvest, crop residues were always left in the field.

The three different tillage practices were then combined with three N fertilization doses applied in sub-plots of 40 m^2: (1) *N0* fertilization, with no N fertilizer supply; (2) *N* fertilization, with a N dose traditionally supplied in the area (120 kg N ha^{-1} y^{-1}), and (3) *N*+ fertilization, involving a N application dose that was 25% higher than *N* (160 kg N ha^{-1} y^{-1}). The N fertilizer (Urea, 46%) was always split in two applications: 60% of total N amount at tillering stage (BBCH code 21) and 40% at panicle differentiation stage (BBCH code 34). In addition, 30 kg P_2O_5 ha^{-1} y^{-1} (13.2 kg P ha^{-1} y^{-1}) and 100 kg K_2O ha^{-1} y^{-1} (83 kg K ha^{-1} y^{-1}) were applied at tillering stage across all treatments. Sole CL variety (imidazolinone-tolerant) was dry seeded at the seeding rate of 170 kg ha^{-1} between the second and the third decade of May in each year. For all treatments, the fields were flooded with a 5 cm water level at tillering stage, approximately one month after seeding, after the herbicide treatments and the first top-dressing fertilization. Afterwards, field flooding was maintained throughout the cropping season, except for one drainage period at panicle initiation stage to allow for second top-dressing fertilization. After drainage period, water level was raised and kept around 10–15 cm, until the field was drained approximately one month prior to harvest.

Weed control differed between tillage practices. In *NT* glyphosate (1080 g ha^{-1}) was applied before seeding. In all tillage treatments, pendimethalin and oxadiazon were applied together in pre-emergence (770 and 380 g ha^{-1}, respectively) and imazamox and halosulfuron-methyl (34 g ha^{-1} and 30 g ha^{-1} respectively) were applied twice in post-emergence.

4.2.3. Crop yield, yield components and efficiency indices

Grain and straw yields were measured every year with a combine harvester at the end of the growing season (first decade of October) when grains reached a moisture content of about 20%. No data were measured for 2017 due to a strong hailstorm that compromised crop yield. Grain and straw samples were dried to reach a moisture content of 14%, and subsequently ground and analyzed for total N by dry combustion (UNICUBE Elemental Analyzer, Elementar, Germany). Moreover, yield components (i.e. panicle density, number of spikelets per panicle, 1000-grain weight and panicle sterility) were measured using a sample of rice plants collected in three 0.25 m^2 areas in each sub-plot before harvesting. Plant density was estimated at seedling emergence stage in three sampling areas (0.25 m^2) for each sub-plot. Tillering capacity index was calculated as ratio between panicle density (at harvest) and plant density (at seedling emergence stage). Apparent Nitrogen recovery

(ANR) was calculated for *N* and *N*+ treatments according to Zavattaro et al. (2012):

$$ANR = \frac{(N\,uptake_N) - (N\,uptake_0)}{F_N} \times 100\%$$

where $N\ uptake_N$ is plant (grain + straw) uptake expressed as kg N ha^{-1} for *N* and *N*+ rate fertilization, $N\ uptake_0$ is plant uptake expressed as kg N ha^{-1} in the *N0* treatment, FN is the amount nitrogen applied with mineral fertilizer (as kg N ha^{-1}). N uptake was obtained by multiplying grain and straw dry weight by respective N content.

4.2.4. Soil measurements

Soil measurements were performed at the end of experimental period (after harvesting in 2019). These measurements were carried out for the three tillage methods and only for one level of nitrogen fertilization (*N* treatment). Soil samples were obtained from subplots where a dose of 120 kg N ha^{-1} was applied, because this is the usual rate applied by local farmers. Two sampling depths were considered: 0–15 and 15–30 cm. The samples were air dried, ground and sieved at 2 mm. SOM characterization was determined by the density fractionation method (Golchin et al., 1994; Sohi et al., 2001), modified to obtain an additional coarse particulate OM fraction (POM) with size > 200 µm. This fraction was obtained by wet sieving 24 g of ground soil (<2 mm) together with 6 stainless steel balls with a diameter of 6 mm, in a rotating sieve (200 µm mesh) immersed in 0.8 L of water for 60 min to facilitate the breakdown of soil macroaggregates and release of coarse free POM. On the fraction obtained after this process (<200 µm) the density-based separation scheme (density cutoff = 1.6 g cm^{-3}; microaggregate breakdown energy =

440 J ml^{-1}) was applied (Golchin et al., 1994). Four fractions were thus separated: (i) free particulate organic matter with dimensions >200 μm (*coarse fPOM*), (ii) free particulate organic matter with dimensions <200 μm (*fine fPOM*), (iii) physically protected intra-micro-aggregate particulate organic matter (*iPOM*); (iv) mineral-associated and chemically protected organic matter (*MOM*). Total soil organic C (SOC), total N (SN) and their distribution between different SOM fractions were determined by dry combustion (UNICUBE Elemental Analyzer, Elementar, Germany).

Soil bulk density was measured in 2019 in *N* treatments sub-plots at a soil depth of 7.5 cm (representative of first layer 0–15 cm) and 22.5 cm (representative of second layer 15–30 cm) using cylinders of volume equal to 100 cm^3, replicated three times for each layer. Dry weight was determined at 105 °C until a constant weight was reached.

The stocks of total SOC, TN and each SOM fractions were calculated as follows according to (Morgan and Ackerson, 2022):

$$STOCK = X \times BD \times H \times 0.1$$

where, X is organic C or N concentration (mg g$^{-1}{}_{soil}$), BD is bulk density (g cm^{-3}); H is soil depth (cm), 0.1 is the conversion factor to obtain value expressed as Mg ha^{-1}.

4.2.5. Data analysis

Yield and yield components data were analyzed by a linear mixed effect (lme) model including tillage practices, fertilization treatments and year as fixed factors and block as random effect. The effects of tillage practices, depth and their interactions on soil bulk density, SOC and SN stocks, C and N stocks in all soil organic matter fractions were tested by two-way ANOVA. Treatment averages were separated through Bonferroni post hoc test at $P<0.05$. Analyses were performed using *nlme*, *emmeans* and *multcomp* R packages.

For multivariate analysis, the PCA was applied by means of the R software library *FactoMinerR.* PCA was performed only on the different tillage methods considering the *N* fertilization level, because previous statistical analysis did not identify significant differences in grain yield between nitrogen levels, except for *N0.* Statistical analysis was performed using R software, version 3.6.2.

4.3. Results

4.3.1. Grain and straw yield

Both tillage and N fertilization significantly influenced rice grain yield, separately and in interaction with year, but the interaction between the two factors was never significant (Table 4.1). *CT* and *MT* never showed differences between them. Conversely, *NT* resulted in a significantly lower grain yield than *CT* and *MT* except for 2014. Looking at the entire period, *NT* average yield was 15% lower than *CT* and *MT*. However, tillage × year interaction in grain yield highlighted a different behavior over time among the three tillage techniques, as *NT* performed not dissimilarly to *CT* and *MT* in 2014 only. The grain yield gap respect to *CT* was more stable over time in *MT* than *NT* (Fig. 4.2). *NT* yield losses increased during the first three years. Successively, a progressive decrease was detected, although the gap was maintained around 1.5 Mg ha^{-1} y^{-1} respect to *CT*. On the contrary, *MT* never showed a yield gap with respect to *CT* except for 2016.

Fertilization did not show any grain yield differences between *N* and *N+* treatments and no significant interaction between tillage and fertilization was evidenced. Straw and grain yield showed a similar behavior. *CT* and *MT* demonstrated higher straw production than *NT*, except in the first year.

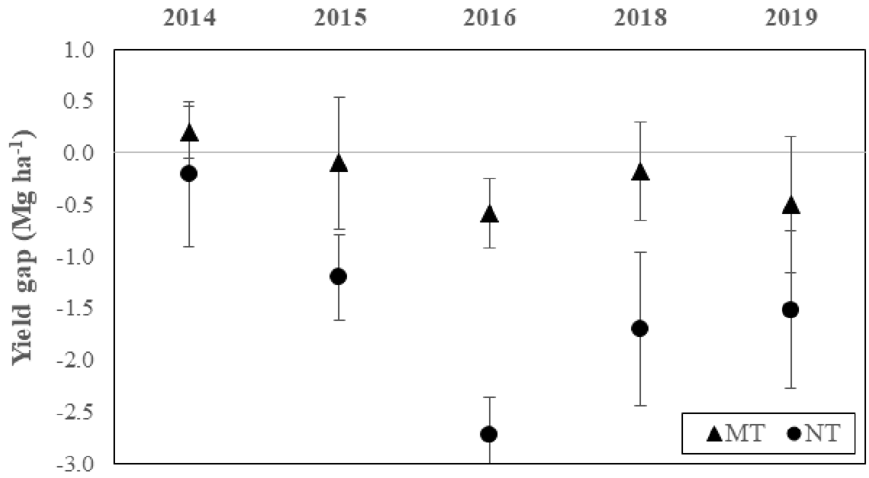

Fig. 4.2. Yield gap (average of different N treatment) expressed as differences among MT and NT respect to CT. Bars indicate standard deviation. CT: conventional tillage with ploughing; MT: minimum tillage with non-inversion surface; NT: no tillage with sod seeding.

Table 4.1. Grain and straw yield (Mg ha^{-1} at 14% moisture) from 2014 to 2019. Values followed by different letters denote differences between treatments (tillage or fertilization) within year (P(f)<0.05).

			2014		2015		2016		2018		2019		Average	
Grain yield (Mg ha^{-1})	Tillage	CT	8.4	a	10.0	a	10.2	a	8.8	a	10.0	a	9.5	a
		MT	8.6	a	9.9	a	9.7	a	8.6	a	9.5	a	9.3	a
		NT	8.2	a	8.8	b	7.5	b	7.1	b	8.5	b	8.0	b
	Fertilization	N+	9.6		10.4		10.1		9.0		10.1		9.8	a
		N	9.3		10.2		9.7		8.5		10.0		9.5	a
		N0	6.4		8.2		7.7		6.9		8.0		7.4	b
P(f)	Tillage: 0.000; Fert: 0.000; Year: 0.000; Tillage*Fert: ns; Tillage*Year: 0.000; Fert*Year: ns													
Straw yield (Mg ha^{-1})	Tillage	CT	9.0	a	10.1	a	8.5	a	9.4	a	9.7	a	9.3	a
		MT	8.8	a	9.8	a	7.8	a	9.2	a	9.1	ab	8.9	a
		NT	7.5	a	7.3	b	5.5	b	7.3	b	8.2	b	7.1	b
	Fertilization	N+	9.7		10.2		8.0		9.4		9.7		9.4	a
		N	9.1		9.5		7.9		9.1		9.7		9.0	a
		N0	6.5		7.6		5.9		7.3		7.5		7.0	b
P(f)	Tillage: 0.000; Fert: 0.000; Year: 0.000; Tillage*Fert: ns; Tillage*Year: 0.046; Fert*Year: ns													

CT: conventional tillage with ploughing, MT: minimum tillage with non-inversion surface, NT: no tillage with sod seeding. N0: no nitrogen applied; N: 120 kg N ha^{-1} year^{-1}; N+: 160 kg N ha^{-1} year^{-1}. Fert: Fertilization

4.3.2. Yield components

Similar to grain and straw yield, for all yield components investigated, the interaction between tillage and fertilization was never found significant (Table 4.2). *NT* showed lower plant density than *CT* in all years, while *MT* was characterized by a variable trend over the years. Panicle density in *NT* management was lower than *CT* except for 2019, in which the three tillage methods resulted in similar values, while *MT* was always similar to *CT*. *NT* management resulted in a higher number of tillers per plant compared to *CT* in 2014 and 2016, in 2015 and 2018 tillering capacity was similar for three tillage methods tested. *NT* resulted in more spikelets per panicle than *MT* and *CT* in three years (2014, 2015 and 2018), while in 2016 and 2019 the three tillage methods provided similar results. *NT* and, in 2016, also *MT* determined a higher 1000-grain weight than *CT*, except for 2014, when no differences among managements were found. *NT* and in 2014 and 2019 also *MT* showed less sterility than *CT*, while in 2015 and 2018 no differences among tillage managements were found. As expected, a N fertilization effect was not recorded on plant density, although panicle density was significantly lower in *N0* with respect to fertilized plots except for 2015, with *N* and *N+* not showing any differences between them. Fertilization × year interaction was not significant for tillering capacity and spikelets per panicle, but the average of five years showed significantly lower values in *N0* with respect to fertilized treatments for both parameters. In contrast, plots not fertilized with nitrogen showed higher values of 1000-grain weight than the fertilized ones. Fertilization with N at both levels resulted in higher sterility than *N0*.

Table 4.2. Yield components (plant density, panicle density, tillering capacity, spikelets per panicle, 1000 grain weight, sterility) from 2014 to 2019. Means followed by different letters denote differences between treatment for each variable (tillage or fertilization effect) within year (P(f)<0.05).

			2014		2015		2016		2018		2019		Average	
Plant density (Plant m^{-2})	Tillage	CT	179	a	359	a	251	a	225	a	257	a	254	a
		MT	126	b	285	b	214	a	219	a	190	b	201	b
		NT	100	b	240	c	118	b	144	b	160	b	158	c
	Fertilization	N+	139		302		197		201		213		211	
		N	132		304		191		192		198		204	
		N0	134		276		192		192		195		198	
P(f)	Tillage: 0.000; Fert: ns; Year: 0.000; Tillage*Fert: ns; Tillage*Year: 0.000; Fert*Year: ns													
Panicle density (Panicle m^{-2})	Tillage	CT	446	a	547	a	464	a	488	a	473	a	484	a
		MT	449	a	575	a	424	a	549	a	418	a	483	a
		NT	382	b	467	b	347	b	406	b	434	a	407	b
	Fertilization	N+	477	a	560	a	427	a	517	a	480	a	492	a
		N	432	a	530	ab	425	a	499	a	483	a	474	a
		N0	368	b	499	b	383	b	427	b	362	b	408	b
P(f)	Tillage: 0.000; Fert: 0.000; Year: 0.000; Tillage*Fert: ns; Tillage*Year: 0.000; Fert*Year: 0.047													
Tillering capacity (Tillers $plants^{-1}$)	Tillage	CT	2.5	b	1.5	a	1.8	b	2.2	a	1.8	b	2.0	b
		MT	3.5	a	2.0	a	1.9	b	2.5	a	2.7	a	2.6	a
		NT	3.9	a	1.9	a	3.0	a	3.0	a	2.3	ab	2.9	a
	Fertilization	N+	3.6		1.9		2.3		2.7		2.4		2.6	a
		N	3.6		1.8		2.4		2.8		2.6		2.6	a
		N0	2.9		1.9		2.2		2.3		1.9		2.2	b
P(f)	Tillage: 0.000; Fert: 0.009; Year: 0.000; Tillage*Fert: ns; Tillage*Year: 0.001; Fert*Year: ns													
Spikelets per panicle (n°)	Tillage	CT	135	b	149	ab	151	a	107	b	136	a	135	b
		MT	139	b	138	b	149	a	109	b	149	a	137	b
		NT	168	a	160	a	159	a	127	a	135	a	150	a
	Fertilization	N+	155		159		160		120		145		148	a
		N	151		154		161		118		141		145	a
		N0	135		133		138		104		134		129	b
P(f)	Tillage: 0.005; Fert: 0.000; Year: 0.000; Tillage*Fert: ns; Tillage*Year: 0.000; Fert*Year: ns													
1000 grain weight (g)	Tillage	CT	24.8	a	24.9	b	23.7	b	24.8	b	24.3	b	24.5	b
		MT	24.9	a	25.2	ab	24.5	a	24.9	b	24.4	b	24.8	b
		NT	24.6	a	25.5	a	25.0	a	25.7	a	25.3	a	25.2	a
	Fertilization	N+	24.3	b	24.7	b	24.1	b	24.5	b	24.1	b	24.3	b
		N	24.6	b	25.0	b	24.0	b	24.8	b	24.3	b	24.5	b
		N0	25.5	a	25.9	a	25.0	a	26.1	a	25.7	a	25.6	a
P(f)	Tillage: 0.001; Fert: 0.000; Year: 0.000; Tillage*Fert: ns; Tillage*Year: 0.000; Fert*Year: 0.038													
Sterility (%)	Tillage	CT	20.9	a	12.4	a	14.9	a	9.7	a	13.0	a	14.2	a
		MT	16.0	b	12.0	a	13.2	a	9.4	a	9.8	b	12.1	b
		NT	13.3	b	10.0	a	7.6	b	9.7	a	8.4	b	9.8	c
	Fertilization	N+	18.5		14.0		13.2		10.2		12.2		13.6	a
		N	18.3		11.8		13.0		11.4		10.9		13.0	a
		N0	13.5		8.7		9.3		7.2		8.2		9.4	b
P(f)	Tillage: 0.000; Fert: 0.000; Year: 0.000; Tillage*Fert: ns; Tillage*Year: 0.000; Fert*Year: ns													

CT: conventional tillage with ploughing, MT: minimum tillage with non-inversion surface, NT: no tillage with sod seeding. N0 no nitrogen applied; N 120 kg N ha^{-1} $year^{-1}$; N+ 160 kg N ha^{-1} $year^{-1}$. Fert: Fertilization

The Principal Component Analysis (PCA) of grain yield components allowed to obtain a set of uncorrelated PCs (Table 4.3). According to Kaiser's rule (Kaiser, 1960), the first two PCs were retained, as they recorded eigenvalues higher than 1 and explained 68.2% of the total variance (36.7% and 31.5% of the total variability explained by PC1 and PC2, respectively). The PC1 had the largest positive correlation with plant density and panicle density and was negatively correlated with tillering capacity. The PC2 showed positive correlation with spikelet number per panicle and sterility and negative correlation with 1000 grain weight. The datapoints referred to *NT* and *CT* management led to two distinct groups that differed mainly along PC1 axis and for higher PC2 values (Fig. 4.2). On the contrary, data referred to *MT* management grouped on an intermediate area of the graph, suggesting that the effect of yield components was weaker than in *NT* and *CT*.

Table 4.3. Results of the Principal Component Analysis (PCA). The table shows the variable loadings, the eigenvalues and the percentage of variance explained for each component.

	PCA Factor				
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Plant density	0.639	-0.197	-0.146	-0.040	0.109
Tillering capacity	-0.483	0.321	0.525	-0.021	0.190
Panicle density	0.482	0.135	0.572	-0.115	0.529
Spikelets number per panicle	-0.103	0.481	-0.606	-0.254	0.571
1000 grain weight	-0.251	-0.549	-0.080	0.547	0.574
Sterility	0.230	0.554	-0.049	0.788	-0.127
Eingenvalue	2.2	1.9	1.0	0.5	0.4
% variance explained	36.7	31.5	16.6	7.9	7.3

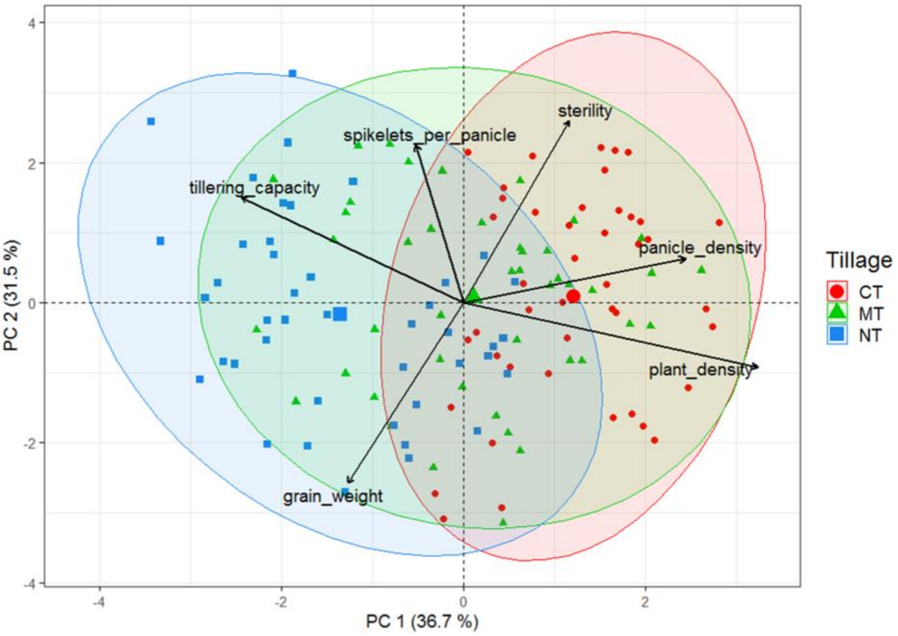

Figure 4.2. PCA Biplot graph based on log-transformed data of grain yield components. CT: conventional tillage with ploughing, MT: minimum tillage with non-inversion surface, NT: no tillage with sod seeding.

4.3.3. N plant uptake and apparent N recovery (ANR)

Total N uptake in *CT* plots was always the highest, while that in *NT* plots the lowest among the tillage managements, except for the first year (Tables 4.4 and 4.5). *MT* did not reduce total N uptake with respect to *CT*, except for 2019. Regarding fertilization management, total N uptake was found to decrease in the order *N+>N>N0*. As expected, tillage × fertilization interaction recorded the lowest values for *N0* in *NT* and the highest for *N+* and *N* in *CT* and *MT* management. Moreover, in *N0* plots total N uptake was lower in *NT* respect to *CT* and *MT*. Straw N uptake also indicated a lower N availability in *NT* and *N0*. ANR showed lower values in *NT* in all years, but in 2014 and 2018, it did not exhibit significant differences compared to *CT*. In 2015, 2016, and 2019, it also did not display significant differences compared to *MT*. Considering the five-year average, ANR was the lowest in *NT* (28.7%) and the highest in *CT* and *MT* (51.4% and 51.1%, respectively). Fertilization did not show a significant effect on ANR.

Table 4.4. Total N uptake and straw N uptake (kg N ha^{-1}) and ANR (Apparent Nitrogen Recovery) from 2014 to 2019. Means followed by different letters denote differences between treatment for each variable (tillage or fertilization effect) within year (P(f)<0.05). Total N uptake means followed by different letters in Tillage × Fertilization effect denote differences between all treatments.

			2014	2015	2016	2018	2019	Average	Average for Tillage*Fertilization N+	N	N0
Total N uptake (kg N ha-1)	Tillage	CT	185.2 a	192.9 a	170.5 a	174.2 a	209.9 a	186.6 a	221.2 a	202.9 ab	135.6 d
		MT	183.5 a	186.6 a	152.5 a	178.3 a	178.0 b	175.8 a	207.5 a	193.3 ab	126.6 d
		NT	164.5 a	138.9 b	108.1 b	126.1 b	160.3 b	139.6 b	158.7 bc	148.4 cd	111.6 e
	Fertilization	N+	201.4	206.6	167.9	190.8	212.3	195.8 a			
		N	194.2	180.8	157.0	170.6	205.1	181.5 b			
		N0	201.4	131.1	106.3	117.2	130.7	124.6 c			
P(f)	Tillage: 0.000; Fert: 0.000; Year: 0.000; Tillage*Fert: 0.023; Tillage*Year: 0.000; Fert*Year: ns										
Straw N uptake (kg N ha^{-1})	Tillage	CT	82.7	80.5	55.5	71.7	81.0	74.3 a			
		MT	81.7	81.2	50.2	74.1	66.5	70.7 a			
		NT	67.0	51.1	33.9	52.0	62.0	53.2 b			
	Fertilization	N+	81.0 a	89.4 a	54.9 a	79.1 a	83.4 a	77.6 a			
		N	81.7 a	69.1 a	50.9 a	69.5 a	79.5 a	70.1 a			
		N0	68.7 b	54.4 b	33.8 b	49.2 b	46.6 b	50.5 b			
P(f)	Tillage: 0.001; Fert: 0.000; Year: 0.000; Tillage*Fert: ns; Tillage*Year: ns; Fert*Year: 0.022										
ANR (%)	Tillage	CT	38.4 ab	52.6 a	50.2 a	42.0 ab	73.6 a	51.4 a			
		MT	48.8 a	39.4 ab	46.4 ab	63.3 a	57.5 ab	51.1 a			
		NT	29.7 b	27.8 b	17.3 b	30.5 b	38.4 b	28.7 b			
	Fertilization	N+	37.5	44.4	36.3	46.0	51.0	43.0			
		N	40.4	35.5	36.2	44.5	62.0	43.7			
P(f)	Tillage: 0.007; Fert: ns; Year: 0.002; Tillage*Fert: ns; Tillage*Year: 0.046; Fert*Year: ns										

CT: conventional tillage with ploughing, MT: minimum tillage with non-inversion surface, NT: no tillage with sod seeding. N0 no nitrogen applied; N 120 kg N ha^{-1} year^{-1}; N+ 160 kg N ha^{-1} year^{-1}. Fert: Fertilization

4.3.4. Soil measurements

Bulk density in the 0–15 cm layer was higher in *NT* than in *CT* and *MT* (Table 4.5). Differences were smaller and non-significant in the 15–30 cm layer, with an average value of 1.56 Mg m^{-3}. Moreover, bulk density increased with depth in *CT* and *MT*, but decreased in *NT*. At the end of the 6-yr experimental period (in 2019), both *MT* and *NT* did not increase total SOC stock in the 0–15 cm soil layer compared with *CT* (Table 4.5). In the 15–30 cm layer, SOC stock was higher in *CT* than in *MT* and *NT*. Observing the differences between depths in each tillage practice, total SOC stock in *CT* management was similar in both layers, while both *NT* and *MT* determined a different distribution of SOC in the soil profile, resulting in its accumulation near the soil surface. A significant tillage × depth interaction was observed in the organic C stocks of SOM fractions, except in the *MOM* fraction (Table 4.6). In contrast to *CT* and *NT*, *MT* showed a higher content of *coarse fPOM* in surface layer than in deeper one. Looking at the 0–15 cm layer, *coarse fPOM* C stock was lower in *NT* respect to *CT* and *MT*. In *CT* management, organic C in *fine fPOM* was more abundant in the deeper layer, while it was homogenous between layers both in *MT* and *NT*. Both *MT* and *NT* showed a higher *iPOM* organic C content in topsoil than in the subsoil, while in *CT* there were no significant differences in *iPOM* across different depths. Evaluation of soil TN and N stocks in the different SOM fractions mirrored organic C behavior. In the 0–15 cm layer *CT* showed a lower N content compared to *MT* and *CT*, while in 15–30 cm the opposite occurred (Table 4.5). The tillage × depth interaction for N stocks of SOM fraction is significant (Table 4.5). In contrast to *CT*, *MT* and *NT* showed a decrease in N stocks in the *coarse fPOM* in the deeper layer, while in *CT* there were no significant differences across different depths. In *MT* and

NT management, N stock in *fine fPOM* was more abundant in the upper layer, while the opposite was true in *CT*. Both *MT* and *NT* showed a higher N stock in *iPOM* in topsoil than in the subsoil, while in *CT* there were no significant differences.

Table 4.5. Total Soil Organic Carbon (SOC) and Total Nitrogen (TN) stocks and soil bulk density measured at 0–15 and 15–30 cm layers at the end of experimental period. Lowercase letters denote different means (P<0.05) between depths for each tillage management, while means followed by capital letters denote different means (P<0.05) between tillage managements within each depth.

Tillage	SOC stock (Mg C ha^{-1})		TN stock (Mg C ha^{-1})		Bulk density (Mg m^{-3})	
	Depth (cm)					
	0-15	15-30	0-15	15-30	0-15	15-30
CT	27.26 A a	27.68 A a	2.94 A a	3.35 A a	1.51 B	1.55 A
MT	30.77 A a	21.23 B b	3.37 A a	1.21 B b	1.53 B	1.55 A
NT	29.15 A a	21.96 B b	2.02 B a	1.39 B a	1.60 A	1.57 A
P(f)						
Tillage	ns		ns		ns	
Depth	0.008		0.007		ns	
Tillage*Depth	0.045		0.041		0.039	

CT: conventional tillage with ploughing, MT: minimum tillage with non-inversion surface, NT: no tillage with sod seeding.

Table 4.6. Distribution of C (a) and N (b) stocks between soil organic matter fractions. Lowercase letters denote different means (P<0.05) between depths for each tillage management, while means followed by capital letters denote different means (P<0.05) between tillage managements within each depth.

	Tillage	***Coarse fPOM***		***Fine fPOM***		***iPOM***		***MOM***	
		Depth (cm)							
		0-15	15-30	0-15	15-30	0-15	15-30	0-15	15-30
Mg C ha^{-1}									
	CT	2.9 A a	3.35 A a	0.30 A b	0.45 A a	1.68 AB a	1.80 A a	22.34	22.08
	MT	3.37 A a	1.21 B b	0.38 A a	0.28 A a	2.28 A a	1.06 A b	24.75	18.68
	NT	2.02 B a	1.39 B a	0.38 A a	0.30 A a	1.48 B a	0.99 A b	25.27	19.31
P(f)	Tillage	0.044		ns		ns		ns	
	Depth	0.003		ns		0.000		ns	
	Tillage*Depth	0.002		0.014		0.001		ns	
Mg N ha^{-1}									
	CT	0.25 A a	0.25 A a	0.02 A b	0.03 A a	0.10 A a	0.11 A a	2.71	2.63
	MT	0.26 A a	0.15 A b	0.03 A a	0.02 A a	0.14 A a	0.08 A b	3.04	2.35
	NT	0.23 A a	0.16 A b	0.03 A a	0.02 A b	0.10 A a	0.06 A b	3.09	2.42
P(f)	Tillage	ns		ns		ns		ns	
	Depth	0.004		ns		0.001		0.012	
	Tillage*Depth	0.041		0.006		0.006		ns	

4.4. Discussion

4.4.1. Effects of conservation tillage on grain yields and yield components

The application of conservative tillage in temperate Italian paddy fields determined different productive results depending on the tillage intensity adopted. This study confirmed that similar rice yields compared to conventional ploughing can be obtained with the adoption of minimum tillage in temperate rice paddies, mainly attributable to a partial straw incorporation and the maintenance of an optimal soil porosity for seed germination (Linquist et al., 2008). In contrast, no tillage led to notable yield reductions of about 15% compared to conventional tillage, similar to what has been already observed in Italy in a silty-loam paddy soil (Cordero et al., 2017; Perego et al., 2019), with significant inter-annual variability in the yield gap over the 6-yr experimental period. In particular, the yield lowered with respect to conventional tillage over the first years of no tillage adoption, but then stabilized after a few years of continuous application. This phenomenon related to the long-term adoption of no tillage is well known in scientific literature (Carlos et al., 2022; Pittelkow et al., 2015).

As highlighted by the PCA, the yield components that determined the highest grain production with ploughing are plant density and consequently panicle density. Indeed, conventional tillage represents the soil management which is able to ensure the presence of better conditions for germination and seedling establishment (Huang et al., 2012). PCA showed also that plant density is the main factor that penalized no tillage.

Low plant density and the consequent yield losses under no tillage are due to increased soil compaction according to Naresh et al. (2016). Our results revealed that the greater soil compaction in the surface layer (bulk density = 1.6 Mg m^{-3} equivalent to a 6% increase) with respect to conventional tillage persisted even after six years of no tillage adoption in paddy soils, suggesting that this soil did not show the self-structuring capacity previously observed by Blanco-Canqui and Ruis (2018). This, together with the alteration of soil physical structure due to flooding conditions (Sacco et al., 2012), is probably due to the high sand (63%) and low clay content (7%) of the soil in the study site. On the other hand, minimum tillage did not determine an increase in soil compaction compared to conventional tillage in line with previous findings (Hu et al., 2007).

Moreover, the uneven seeding depth due to the impossibility of soil levelling and the deep tracks left by harvesting equipment in rice paddies, can also cause uneven germination and poor seedling establishment under no tillage, as has already been previously reported by Kumar and Ladha (2011). In this experiment, the low germination and crop seedling density may also have been due to reduced seed/soil contact, related to the high amount of crop residues on the soil surface. The choice of appropriate seeders, especially if equipped with double disc elements, can limit this problem (Crusciol et al., 2010). In addition, when practicing conservation tillage, it is advisable to consider using rice cultivars with high early vigor (Heinemann et al., 2009).

In conservation tillage plant reacts to the low plant density by producing more tillers per plant, and although the panicle density was lower, the plants produced more spikelets per panicle and larger seeds, as observed by Huang et al. (2015). On the other hand, in conventional tillage the greatest panicle density resulted in the production of smaller panicles and seeds. Our results evidenced that the compensation between yield components, which is common in rice (Huang et al., 2011b), was not sufficient to compensate for the lower plant density observed with conservation tillage, especially if the number of seedlings was too low. The lower production of no tillage can therefore be the result of a series of effects, in particular the poor seedling establishment due to low uniformity of seed germination, which eventually leads to a low plant density, as also found by Mohanty and Painuli, (2004).

The absence of an interaction between tillage and fertilization for all parameters indicates that the yield deficit obtained with no tillage cannot be recovered by increasing N fertilization even though this still determined a response from the plant as evidenced by the greater plant N uptake. Other studies pointed out that an increase in N fertilizer is not sufficient to compensate for a lower production due to a low plant density (Huang et al., 2013).

4.4.2. Effects of conservation tillage on N cycling and apparent N recovery

Conservation tillage practices are known to influence both the availability and plant uptake of N, by affecting the input and turnover of crop residue N, the fate of fertilizer N, as well as seedling establishment, root development and temporal changes in crop N requirements during plant growth (Huang et al., 2012).

Rice straw residues generally contain about 70 kg N ha^{-1} thus acting as an important source contributing to soil N pools, and possibly serving as a potential source of available N for the subsequent crop (Zavattaro et al., 2008). Although tillage practices did not significantly affect straw N contents, straw yields and consequently residue N inputs were significantly affected by conservation practices, in particular by no tillage. In fact, straw N uptake at harvest was around 20 kg N ha^{-1} less under no tillage practices with respect to minimum or conventional tillage (71 and 74 kg N ha^{-1}, respectively), despite the higher TN stock in *NT* in the 0–15 cm layer Moreover, unlike inorganic N fertilizer, the release of N from crop residues is closely linked to their decomposition, which, in turn, is influenced by their chemical composition, placement in the soil (e.g. incorporated into the soil or let on surface); additionally, overall environmental conditions play an important role (Cucu et al., 2014). We hypothesized that the lower straw N inputs together with the reduced mineralization of crop residues (and release of plant available N) that are left on the soil surface under no tillage with respect to conventional or minimum tillage where the residues are incorporated into the soil, are responsible for the lower *coarse* and *fine fPOM* N contents observed in the subsoil after 6 years, and could explain the decreasing trend in plant N uptake over time in the unfertilized plots under no tillage. Instead, the higher N stocks in the iPOM in both conservative tillage methods may be related to the improved stability of soil aggregates that typically occurs with these tillage practices (Topa et al., 2021).

Notwithstanding the variability in ANR over the years in the different tillage managements, probably triggered by the inter-annual variability in climatic conditions (Ando et al., 2000), the adoption of no tillage practices generally resulted in the lowest ANR values. The ANR decrease in NT with respect to the other tillage practices corresponded to a lower N uptake. The low N uptake was probably related to lower straw and grain production and to lower root development in compact soil in the early vegetative stages under no tillage (Huang et al., 2012). Moreover, sod seeded rice is known to be characterized by a higher N absorption after heading (Huang et al., 2016), and this could influence the synchrony between fertilizer N supply and plant N uptake. In fact, Huang et al. (2015) reported that the negative effects of sod seeding on N absorption could be partially mitigated by postponing N fertilization. Due to the excessively low plant density with no tillage, increasing N fertilization did not result in a positive effect on ANR, but it probably increased N immobilization and losses. In fact, with the presence of crop residues with a high C:N ratio in the superficial soil layer, microbially-mediated processes could be responsible for the immobilization of 27-50% of applied N (Said-Pullicino et al., 2014), as confirmed by the higher TN stock in *MT* and *NT* in the superficial layer compared with *CT*.

4.4.3. Effects of conservation tillage on SOM pools and SOC stocks

Adoption of conservation tillage practices for 6 years in rice paddies determined a significant stratification of SOC rather than a difference in the total SOC stocks, in line with several other findings reported for other cropping systems (Abdollahi at al, 2017; Rounak et al., 2022). Most of this depth differentiation was due to management induced changes in particulate SOM fractions, as the most stable mineral-associated OM fraction that comprised about 84% of total SOC, did not show significant tillage-induced differences in C stratification. There are conflicting results in the literature regarding the capability of conservation agriculture to increase soil C stocks and soil fertility.

Fangueiro et al. (2017) reported an increase in SOC after 7 years of no tillage adoption compared to conventional tillage in a loam paddy soil, but according to these authors, the SOC increase is more relevant in semi-arid environmental conditions and in soils with low organic matter content. Probably in this experimental site, characterized by a temperate climate and sandy soil with a high organic matter content, six consecutive years of application were not sufficient to determine an increase in C.

The stratification of soil properties, particularly the distribution of organic C resulting from conservation tillage, could be attributable to two main factors, as noted by Blanco-Canqui and Lal (2008) and Shang et al. (2021): the accumulation of crop residues on the soil surface and the reduction of soil disturbance. However, paddy management itself may compromise aggregate stability with flooding due to the disruptive energy occurring upon slaking (Six et al., 2000), and reductive dissolution of Fe-mineral binding agents holding aggregates together

(Giannetta et al., 2022), therefore partially counteracting the benefits on conservation practices on soil structure. Our results nonetheless evidenced that the adoption of minimum tillage in rice paddies led to the highest amounts of labile coarse and physically protected POM in the superficial soil layer and induced significant stratification with respect to both conventional and no tillage practices. The accumulation of labile OM in the topsoil can represent an important source of nutrients for the crop in the rooting zone through decomposition, and is known to contribute to aggregate stability and soil structure favoring the physical stabilization of OM (Wang et al., 2012). In fact, it can be hypothesized that the presence of high amounts of POM in the surface horizons under minimum tillage could promote microbial activity that contributes to the formation and stabilization of water-stable aggregates, that can in turn serve to further SOM stabilization processes within microaggregates having a high mechanical stability (Bucka et al., 2021). In contrast, the lower OM inputs under no tillage confirmed by the lower straw yields were probably responsible for the lowest amounts of labile and physically protected OM with respect to the other tillage practices. Although in the long term the accumulation of OM in the superficial soil layer is known to contribute to limiting soil compaction in no tillage (Blanco-Canqui and Benjamin, 2013), the negative effects of this tillage practice on crop yields actually limits the topsoil OM contents in these paddy soils where crop residues represent the only OM inputs. In this light, in order to enhance the positive effects of no tillage on paddy soil properties, this practice should be combined with complementary techniques, such as the use of cover crops, to further increase the OM inputs and promote soil aggregate stability (Blanco-Canqui and Ruis, 2018).

4.5. Conclusions

Conservation tillage in Italian rice cropping system has shown varying effects on rice yield and soil fertility in the medium term. Among the different conservation soil management practices, minimum tillage emerges as the most suitable alternative to conventional tillage. It maintains high yields by using production resources more efficiently and sustains soil fertility by promoting OM and N inputs, while also facilitating soil aggregation and preventing soil compaction. However, the adoption of no tillage in our climatic conditions and cropping system reduces rice yields (–15%). This reduction is primarily caused by reduced plant density due to the presence of crop residues and greater soil surface compaction, which makes the planting operation challenging. This, in turn, leads to inadequate seed-to-soil contact, compromising crop germination and seedling emergence.

Rice plants react with a greater tillering and a higher number of spikelets per panicle, however this is still insufficient to bridge the yield gap compared to conventional tillage. Additionally, no tillage tends to reduce ANR, and increasing the amount of mineral N supplied is not enough to compensate for the yield gap due to the low plant density. In fact, this practice may even lead to increase N losses.

Considering these factors, no tillage is not suitable for rice cultivation in Italian temperate rice fields. In paddy soils under temperate climate, conservation tillage did not lead to an evident improvement in the soil physical-chemical fertility in the medium term. However, some positive effects were observed, primarily limited to the surface soil layer.

5. General conclusions

The sustainable increase of rice production for food security requires efforts to enhance the capacity of rice production systems to adapt to global climate change as well as to mitigate the effects on global warming. In this context, the aim of this thesis was to evaluate, for Italian temperate rice cropping systems, the viability of new techniques to mitigate the negative effects of conventional practices on climate change and at the same time face the limitation of resources (such as water) imposed by climate change. The research on innovative rice cultivation practices aimed at reducing the impact of climate change is constantly evolving because today farming systems have more obvious and detectable social, ecological, economic and environmental implications than ever before, including growing concerns regarding their agricultural and environmental sustainability. However, the sustainable intensification of the rice systems can only be achieved if the promotion of more environmentally friendly practices is supported by evidence on the agronomic and economic sustainability without neglecting any important trade-offs that could occur.

The field experiment described in Chapter 2 was carried out to meet the first objective of this thesis. This study demonstrates that the environmental impact of conventional continuous flooding in Italian temperate rice systems can be mitigated through the adoption of water-saving techniques such as AWD while maintaining similar or improved agronomic performance. Alternate wetting and drying could be a workable solution for sustainable and environmentally friendly rice cultivation in Northern Italy and potentially in the rest of southern Europe, even in the context of adaptation to the ever limiting availability of water resources. Indeed, this study confirmed the high potential of AWD in reducing CH_4 emissions without increasing N_2O emissions, thereby resulting in a reduced GWP. Despite these potential benefits, our results also showed that there are important trade-offs related to food safety that need to be taken into consideration when adopting AWD. In particular, although AWD was found to be an appropriate strategy to reduce rice grain As concentrations, a contemporary increase in Cd and Ni contents to levels that sometimes exceeded the limits adopted by the European Commission, may be of concern for food safety and require the identification and adoption of specific abatement measures.

The findings of Chapter 2 also showed that under field conditions AWD did not negatively affect N uptake and N apparent recovery at the end of the cropping season. However, as highlighted by key question 3 and objective 2 of this thesis, providing adequate N supply for rice plants, particularly during the early vegetative stages, is one of the most pressing challenges under AWD management. Therefore, the mesocosm experiment reported in Chapter 3 was carried out to underline the mechanisms controlling N availability and plant uptake as a function of water, straw and fertilizer management practices. The findings reported in this Chapter provide important insights that allow to better understand the complex interactions between water, fertilizer and crop residue management that influence N supply from different sources. Moreover, it highlights the need to specifically optimize these interactions to improve N uptake by plants. Although AWD reduced total N uptake by about 4-25% with respect to continuous flooding over the first phases of rice growth, this could only be partly attributed to a lower uptake of fertilizer-N (and lower fertilizer-N use efficiency), suggesting that other N sources were affected by water management. In fact, the interaction between soil redox conditions (i.e. water management) and the availability of labile C (i.e. crop residue management) and inorganic N (i.e. fertilization management) strongly determined the supply of soil-derived N through microbial feedback and priming responses. Although incorporated straw contributed only minimally to rice N, it represented the primary driver controlling plant N nutrition through these microbial responses. These insights contributed to identify suitable fertilization practices that favour plant N uptake during the early stages of rice growth under AWD. An equal splitting of N fertilizer between pre-seeding and tillering with a late incorporation of crop residues (i.e., about 30 days before seeding) improves N nutrition of rice when grown with water seeding and AWD irrigation. Although this study highlighted how management practices may modulate the contribution of different N sources to plant nutrition, most of these effects are a result of changes in plant–microbe interactions in the rhizosphere that are not always unequivocal. Several questions regarding root–shoot and root–soil interactions and N losses via ammonia volatilization, nitrification, and denitrification under water-saving irrigation and the mechanism involved, still remain unanswered. This warrants further research to understand how these interactions are infuenced by changes in soil redox conditions and their implications on plant nutrition, in order to provide useful indications for N management in rice paddies.

Although Chapters 2 and 3 provide useful evidence that supports the adoption of AWD as a viable alternative to continuous flooding to improve agro-environmental sustainability of temperate rice cropping systems, the widespread adoption of AWD in Italy and across Mediterranean area is still constrained and primarily limited by the lack of information on AWD management at large scales and the risk of yield losses in case of improper management. Increased efforts need to be made to up-scale results from field to irrigation district scales where additional constraints may be encountered. Therefore, the application of these outcomes at larger scales (e.g., irrigation district, catchment) requires further considerations. For example, the applicability of the different water management techniques may depend on the water availability and peculiarities of the irrigation system. Based on these considerations, the outcomes of this work can be also useful at larger spatial scales, in districts that are predominantly cropped with rice in monoculture, where the knowledge of the advantages and disadvantages of each management practice can support the selection of the most appropriate management for the different local conditions. The full benefits of AWD cannot be realistically achieved by indiscriminantly adopting this practice across all rice cropping areas, but an integrated approach is required to protect natural resources (i.e. water and soil) while enhancing the resource use efficiency and overall profitability at farm scale. These challenges require more information on the pedoclimatic and hydrological suitability of different rice farming areas to AWD, the long-term effects of AWD on hydrological budgets at district scale and on soil quality (such as soil C stocks), as well as site-specific technologies for correctly adopting AWD and extension work to improve the farmers' perception.

Another widely recognized technique for mitigating climate change in many cropping systems is conservation agriculture. Objective 3 of this thesis was to evaluate the application of conservation tillage in the Italian rice cropping system and its effects on physical and chemical soil fertility. The study reported in Chapter 4 evidenced that different conservation soil management practices have varying effects on rice yield and soil fertility in the medium term. Minimum tillage emerges as the most suitable alternative to conventional tillage for improving the environmental and economic sustainability of Italian temperate rice area. It maintains high yields by using production resources more efficiently and sustains soil fertility, facilitating soil aggregation and preventing soil compaction. On the other hand, in Mediterranean climatic conditions no tillage is not a viable practice to meet the increasing demand for rice production because it reduces rice yields by about 15% and increases soil compaction. The results of this study show that under conservation tillage the majority of stored SOC occurs in the shallow 0–15 cm, and this effect of SOC stratification at the surface represents a positive ecosystem service. Therefore, to achieve sustainable food production with minimal impact on the soil and the atmosphere, conservation tillage practices become more important now than ever. In the scenario of climate change, it is imperative to promote the adoption of conservation agriculture for the long-term sustainability of the rice sector. However, further validation of this approach requires research and development on suitable tillage implements, evaluation of better land preparation methods under minimum tillage systems and testing in more diverse agro-ecological conditions and soils through on-farm trials.

In conclusion, the introduction of the agronomic techniques tested in this thesis (AWD and conservation tillage), with the specific benefits previously described, can help mitigate the impact of rice cultivation on climate change, while at the same time enable the cropping system to adapt to it. The positive effect of these techniques can be further enhanced if applied in combination with other strategies. For example, winter flooding combined with AWD or minimum tillage can enhance the mitigation effect of atmospheric emissions through the beneficial effects on straw degradation; or N use efficiency with AWD can be improved by using fertilizers with nitrification and urease inhibitor; or the use of cover crops can help increase soil organic matter and soil fertility.

Therefore, the understandings and results obtained in this thesis provide practical implications on innovative management of rice paddies. Altogether it represents a step forward towards the implementation of a more economically and environmentally sustainable rice cultivation in Europe, providing scientific evidence that can also be extended to other temperate rice growing regions in the world. Future work may focus on the verification of these practices in various geographical zones with feasibility in varying circumstances to provide site-specific mitigation packages.

We hope that due to its practical implications, this work will be fruitful in the future not only to motivate and guide further research work, but also to provide support for policy makers and regulatory systems, stakeholders, and for the public at large, to develop and transfer appropriate and efficient technologies, that will be vital for the realization of measures for sustainable rice production.

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