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"Evaluation of sustainable and diverse cropping systems: soil tillage and fertilization within wheat-potato crop rotation and varietal selection of durum wheat"

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PhD Student:
Dott. Verdiana Petroselli

Verdiana Petroselli

PhD Coordinator:
Prof. Roberta Bernini

Tutor:
Prof. Roberto Mancinelli

Co-Tutor:
Prof. Emanuele Radicetti

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SUMMARY

Abstract

Ensuring food security will be one of the biggest challenges that the agriculture industry will be facing in the future globally, considering all the negative repercussions that climate change will have on crops. Current agricultural systems with high inputs and intense use of resources are one of the causes of deforestation, water scarcity, soil depletion, biodiversity loss, resistance to pathogens, and high levels of greenhouse gas emissions, which in turn can no longer guarantee sustainable agri-food production. Sustainable agricultural practices have the potential to reverse the trend of conventional agriculture. The research has allowed the study of sustainable cropping systems (in terms of production and environmental impact) and the evaluation of durum wheat varieties with greater performance concerning management, water availability, and different input levels of fungicides. In particular, two of the world's main crops have been studied, which are the basis of global and Mediterranean nutrition: wheat and potatoes, with world production of 771 million tons and 376 million tons, respectively (data for the year 2021). These were initially studied in a long-term rotation system. The evaluation was carried out by adopting three different soil treatments (plowing, subsoiling, and spading machine) and two different fertilization sources (mineral and organic). During the years of study, the account has been taken of how these reacted to the different thermo-pluviometric trends and how they interfered with the quality and components of the soil, weeds, and productivity. In parallel, the evaluation of durum wheat was explored in depth in a second experiment, in which the possible adaptation strategies to climate change were explained in the testing of 15 varieties which were tested using increasingly technological and universal evaluation protocols, such as VCU (Value for Cultivation and Use). The objective was to assess the performance of varieties and their characteristics with different management approaches to predict the use of more drought-resistant varieties, capable of using water resources more efficiently and adapting better to new environmental conditions while maintaining high nutritional qualities. In particular, performance was evaluated under two different management regimes relating to fungicides and growth regulators (PGR), called Full and Minimal, and under an organic approach and an irrigation regime. The assessments carried out can help to better understand the environmental impacts that agricultural management has on the various components of the system, to choose the best alternatives to enhance production, quality, and the economic-environmental impact.

General Introduction

Overview

Globally, the current state of food security and nutrition shows a worrying picture. The report "The State of Food Security and Nutrition in the World 2023" prepared by the five bodies of United Nations (FAO, IFAD UNICEF, WHO and WFP) reveals that the number of people who suffered from hunger in 2022 is estimated between 691 to 783 million people [1]. With expected population growth the demand and preferences for food are changing. The persistent poverty, inequality and unemployment limits the access to food by hindering the achievement of food and nutrition security objectives. Also, to overcome the problems of scarcity of natural resources like water and limited availability of agricultural land there is an urgency to act on the increasingly tangible impacts of climate change on agri-food systems.

Agriculture is the sector most affected by climate change and global warming because of its enormous size and sensitivity to agro-climatic weather conditions [2,3]. Climate change threatens to reverse the progress made so far in the fight against hunger and malnutrition [4] (Figure 1). Climate change has both direct and indirect impacts on agri-food systems. Direct impacts are due to a change in physical characteristics such as temperature levels and distribution of precipitation, indirect ones are those that affect production through changes on other factors such as pollinators, parasites, disease vectors and invasive species. Some of these impacts can be easily predicted, such as the direct impact of a heat wave on a given species in a given phenological phase. Others are more difficult to predict, such as the effect of a certain climate change on an entire ecosystem because it is made up of more elements that will react and interact differently [4]. Climate change will determine the future state of natural resources and production because it affects countries and sectors differently. It will also change the distribution of income, the endowment of resources and the opportunities for profit. Different social groups and countries show different degrees of vulnerability, depending on their exposure to climate change, the sensitivity of their livelihoods (percentage of income or Gross Domestic Product -GDP-) and their adaptability [5]. The availability of arable land in the future will depend on several biophysical constraints linked with climatic conditions that will either make soil suitable or not, as well as socio-economic development, urban expansion, and conservative management. Currently, about one third of the world's agricultural land is considered moderately to highly degraded. This degradation affects drylands in particular and has a negative impact on the quality of local people's livelihoods and the long-term health of ecosystems. The term “degradation” refers to a deterioration in the biophysical value of the environment that can be caused by natural or anthropogenic processes. Such processes include desertification, salinisation, compaction or invasive species invasion. Globally, only few opportunities are left to further expand agricultural areas. Moreover, in many areas of low rainfall in the Near East, North Africa and Central Asia, farmers have used a large part of the available water resources, resulting in the depletion of rivers and aquifers. However, cultivated areas that lose part of their productive potential are not only abandoned but may also be converted to other uses. Given these limitations in land and water resources, it is likely that in the future the necessary quantities of food will have to be produced mainly through increases in yields, rather than from expansions in cultivated areas. But unfortunately, world's yield growth has slowed down over the past 20 years, and in some regions their maximum potential has already been reached [6,7]. Crop yields are not only determined by technological progress, but are also altered depending on species, genotype and location. By changes in climatic conditions that influence thermal and hydrological regimes, the structure and functionality of ecosystems is also disturbed. For each crop, the effect of temperature change will depend on the optimal temperature of the crop at the time of growth and reproduction. In some areas heating could benefit the types of local crops or crops that are currently grown in warmer areas. However, temperatures too high and higher than optimal ones could damage plant cells and cause decreases in yield. Just as it has been seen that the increase of CO_2 levels can stimulate the growth of some plants, in particular, C3 plants are preferred over C4 ones [8], but if this is associated with poor availability of water and nutrients, increases in atmospheric carbon dioxide can reduce the nutritional value of crops, including the content of essential proteins and minerals [9]. Current agricultural systems with high input and intense use of resources are one of the causes of deforestation, water scarcity, soil depletion, biodiversity loss, resistance of pathogens to pesticides and high levels of greenhouse gas emissions, which in return can no longer guarantee sustainable agri-food production [10]. To enable actors in the agri-food chain to continue producing, transforming, marketing and consuming safe and nutritious food and other products and services requires a range of climate resilience and adaptation actions based on healthy and conservative agroecosystems that at the same time reduce energy losses and consumption [11]. Reducing the vulnerability of the system is crucial to reduce the impact on it. Sustainable agriculture have the potential to reverse the trend of conventional agriculture by minimising soil disturbance, preserving ecosystem biodiversity, emitting lower greenhouse gas emissions, uses less fertilizer and leads to more carbon sequestration in the soil [12]. Transforming agriculture, and placing it on a sustainable path, is the task of agroecology [13].

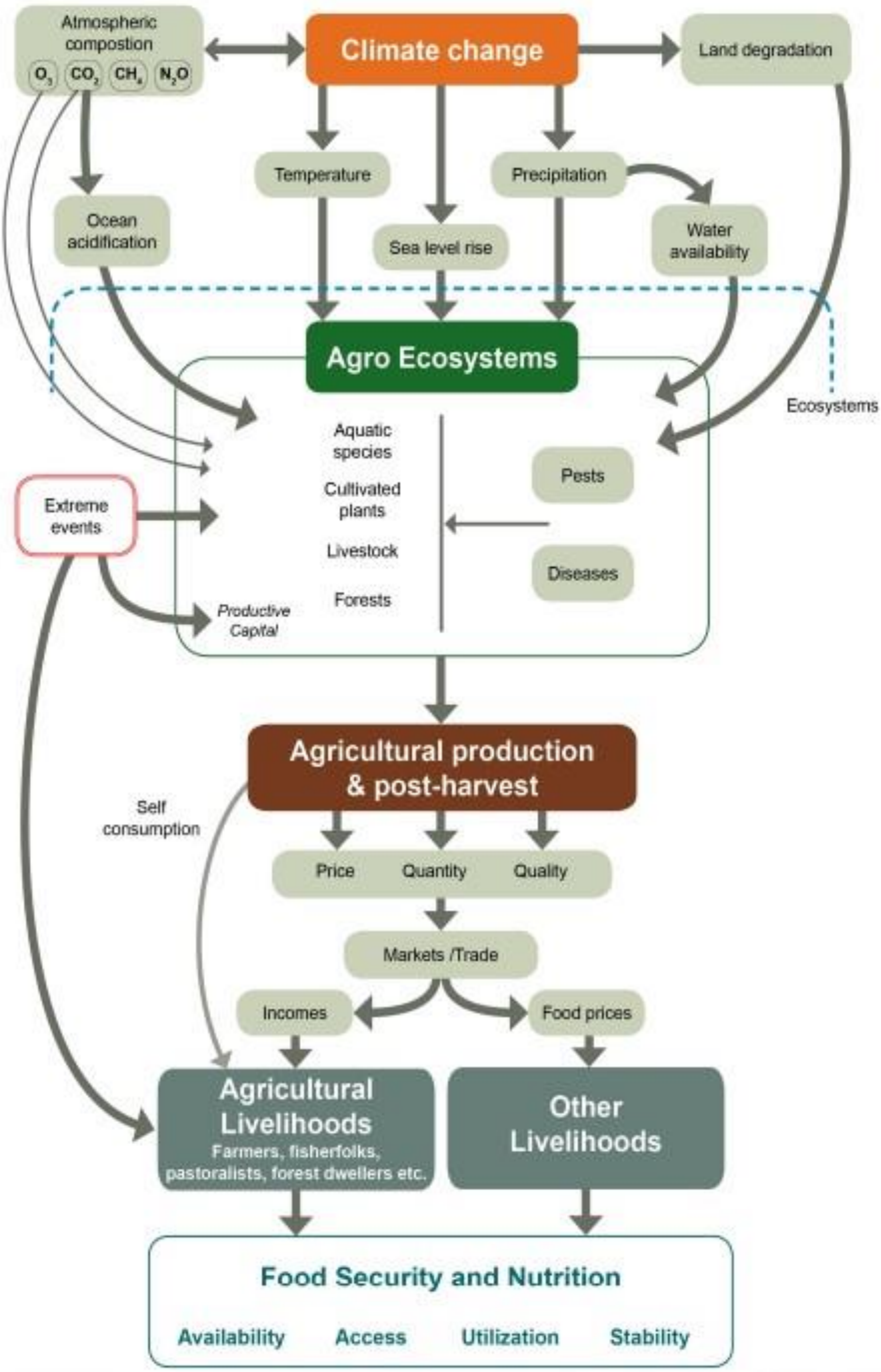

Figure 1. Schematic representation of the cascading effects of climate change impacts on food security and nutrition. Source: FAO, 2015.

A Sustainable Approach: Agroecology

Agriculture is a human activity, created and adapted to meet social needs. Its key role in human life is clear, however, its impact on natural ecosystems is also important [14]. Understanding, assessing, and balancing the positive and negative impacts it has on the environment is an ethical responsibility of human beings [15]. Agricultural sustainability is based on the principle that we must meet the needs of the present without compromising the ability of future generations to meet their own needs [16]. Therefore, the long-term management of natural and human resources is of equal importance to short-term economic gain. The living conditions of workers and rural communities, consumer safety, and the maintenance and improvement of natural resources are equally important, in a holistic and multidisciplinary view of the whole system. To achieve these goals, one must consider how to take advantage of natural and existing processes, relying more on the internal nutrient and energy cycle [17].

Agroecology is the integration of research, education, action, and change that satisfies the sustainability of the food system in all its aspects: ecological, economic, and social. Firstly, agroecology is based on a scientific approach. It improves knowledge of the ecological relationships between species, environment, and natural systems, to design and manage sustainable agroecosystems. Secondly, it has a practical approach. Combines traditional and local agricultural knowledge with innovative practices to find agricultural practices that meet the food needs of the present and future. Moreover, it also has a social impact. It not only supports changes that will lead to food security but also encourages knowledge of how these changes can be implemented [13]. Agroecology deals with energy flows, interactions between species, and the cycle of materials, but also with the stresses on agricultural systems coming from factors outside agriculture. In particular, the global impact of human activity requires that agroecology face a complex, dynamic, and increasingly uncertain context. The focus of agriculture will no longer be just on the field but will be on a landscape, continental, and global scale [18]. Reducing the concept of an "agricultural system" to "crop" implies treating food only as a commodity, and thus as something that can be easily moved away from its place of origin. In contrast, when considering the crop within a system, the complex socio-ecological relationships are also considered [19]. It is also known that in agricultural systems it is biodiversity that provides ecosystem services such as recycling nutrients, controlling the local microclimate, regulating local hydrological processes, regulating the abundance of unwanted organisms, and detoxifying harmful chemicals. If these biological ecosystem services are lost due to system simplification, the result is an artificial system that requires constant human intervention. In this way, the natural dispersal of seeds is replaced by mechanized sowing, the natural controls on weeds, insects, and pathogens are replaced by chemicals, and decomposition is altered with the harvest and recycling of nutrients with the use of fertilizers. Therefore, modern agricultural systems have become productive, but only because they are highly dependent on external inputs [20]. If the modification of natural factors has always related to the exercise of agricultural activity, more recently control interventions are based on the development of scientific knowledge of the soil-climate-plant system and the possibilities of monitoring its parameters to intervene with the appropriate means at the best time. From the concept of the crop, with agroecology, we move to the agroecosystem.

According to Agroecology Europe the fundamental principles of agroecology that provide guidelines for creating agricultural practices that are productive, sustainable and in harmony with the environment are [21]:

1. Recycling. Preferentially use local renewable resources and close as far as possible resource cycles of nutrients and biomass.
2. Input reduction. Reduce or eliminate dependency on purchased inputs.
3. Soil heath. Secure and enhance soil health and functioning for improved plant growth, particularly by managing organic matter and by enhancing soil biological activity.
4. Animal health. Ensure animal health and welfare.
5. Biodiversity. Maintain and enhance diversity of species, functional diversity and genetic resources and maintain biodiversity in the agroecosystem over time and space at field, farm and landscape scales.
6. Synergy. Enhance positive ecological interaction, synergy, integration, and complementarity amongst the elements of agroecosystems (plants, animals, trees, soil, water).
7. Economic diversification. Diversify on-farm incomes by ensuring small-scale farmers have greater financial independence and value addition opportunities while enabling them to respond to demand from consumers.
8. Co-creation of knowledge. Enhance co-creation and horizontal sharing of knowledge including local and scientific innovation, especially through farmer-to-farmer exchange.
9. Social values and diets. Build food systems based on the culture, identity, tradition, social and gender equity of local communities that provide healthy, diversified, seasonally and culturally appropriate diets.

10. Fairness. Support dignified and robust livelihoods for all actors engaged in food systems, especially small-scale food producers, based on fair trade, fair employment and fair treatment of intellectual property rights.

11. Connectivity. Ensure proximity and confidence between producers and consumers through promotion of fair and short distribution networks and by re-embedding food systems into local economies.

12. Land and natural resource governance. Recognize and support the needs and interests of family farmers, smallholders and peasant food producers as sustainable managers and guardians of natural and genetic resources.

13. Participation. Encourage social organization and greater participation in decision-making by food producers and consumers to support decentralized governance and local adaptive management of agricultural and food systems.

One of the most significant tools to facilitate the transition towards agroecology is represented by the "ESR" (Efficiency, Substitution, Redesign) framework created by Hill and MacRae [22]. This model represents a clear roadmap that helps understand how agriculture can evolve from conventional practices to fully sustainable systems through three distinct phases (Table 1).

1. Efficiency. At this stage, the goal is to improve resource efficiency within existing agricultural systems to reduce consumption and waste of expensive and scarce resources. This can include more precise use of chemical inputs, optimizing irrigation techniques, and improving soil management practices.

2. Substitution. This phase involves the replacement of chemical inputs and conventional agricultural practices with more ecological and sustainable alternatives. For example, biopesticides and biological control, i.e. the replacement of chemical pesticides in favor of useful organisms or natural substances; the use of organic fertilizers such as compost, manure, or other natural fertilizers instead of synthetic ones; and Integrated Pest Management (IPM): Combining biological, physical, and cultural methods to control pests.

3. Redesign. Finally, this stage is achieved when the causes of problems are recognized, and therefore prevented, and are resolved internally through site- and time-specific design and management approaches. The system, therefore, obtains greater resources self-reliance and resilience are achieved.

Table 1. Comparison of three approaches to sustainable agriculture. Source: Hill and MacRae, 1996.

UNSUSTAINABLE	SHALLOW SUSTAINABILITY		DEEP SUSTAINABILITY
CONVENTIONAL	**EFFICIENCY**	**SUBSTITUTION**	**REDESIGN**
EXAMPLES			
Factory Farm	Low-Input and Resource Efficient Agriculture	Eco-Agriculture	Permaculture, Natural and Ecological Farming
APPROACHES			
High power, imported, non-renewable	Conservation	Solar and renewable	Integrated use of local inputs, reduced demand
Physical/chemical (soluble fertilizer, pesticides, biotechnology)	Physical/chemical/ biological (slow release, band)	Biological and natural materials	Bio-ecological
Imported input-intensive	Efficient use	Alternative inputs	Knowledge/skill intensive
Narrow focus, farm as factory (linear design and management)	Efficient factory	Benign factory	Broad focus, farm as ecosystem (integrated design and management)
Problems as enemies to eliminate and control directly with products and devices	Efficient control (monitor pest, Integrated Pest Management)	Biocontrols and more benign alternative interventions	Prevention, selective and ecological controls (pests as indicators)
GOALS			
Maximize production (neglects maintenance) over short-term	Maintain production while improving maintenance	Improved maintenance	Optimize production (emphasizes maintenance and system "health") over long-term
Create demand, manipulate wants; global marketing			Meet real needs; mostly local distribution

Agroecological strategies to improve utilization and reduce nutrient losses are all based on positive and negative interactions between biotic and abiotic organisms and processes [23,24]. Conservation tillage, the use of compost, and selection of the genotype are some examples of sustainable agricultural practices. Conservation tillage that aims to achieve sustainability, for example, can have various levels. One can talk about Efficiency (ESR) through minimal soil disturbance (i.e. zero, minimal or stubble tillage) or precision farming; and one can meet the level of Substitution with the use of cropping system diversification, defined by FAO as an approach to agricultural management that increases the variety of crops grown in a given space and time; diversification of cropping systems can include a range of practices such as crop rotation, intercropping, cover crops and agroforestry systems [25]. The potential benefits of these methods lie not only in the conservation but also in the improvement of natural resources (such as increasing soil organic matter) without constituting an obstacle to productivity. This allows the soil to act as a carbon dioxide sink, increase water holding capacity, and reduce erosion [26]. Fertilization, on the other hand, can fall into the Efficiency phase when its application is carried out in optimal quantities and times to reduce waste and improve the efficiency in the use of nutrients (this can also be facilitated using sensors and field maps) and can be replaced (Substitution) with compost, manure or other organic sources of nutrients and beneficial microorganisms. The use of compost, in particular, represents the greatest reliance on local or agricultural renewable resources. Compost has the advantage of improving soil structure and aeration, increasing water retention capacity, and stimulating soil fertility [26,27]. Finally, the practice of genotype selection, which regulates the productivity, sustainability, resilience, and quality of a crop, can also be applied at various levels. Efficient (E) selection involves the choice of varieties that guarantee high yield with the optimal use of available resources and the use of varieties resistant to abiotic stress; while Substitution involves the use of improved varieties through traditional breeding techniques and the use of native varieties that are well adapted to local conditions [28]. As a result, all these conservation practices tend to preserve natural resources and biodiversity [29]. In agricultural systems, the biodiversity level determines the resilience of a system. In agroecosystems, two categories of diversity can be distinguished: functional diversity and response diversity [30]. The former refers to the variety of organisms present and the various ecosystem services they provide, while the latter determines the difference in responses due to various environmental changes. A system is effective and resilient when high species diversity (functional diversity) manifests itself in a high response diversity that allows the ecosystem to function continuously despite changes (Figure 2) [30].

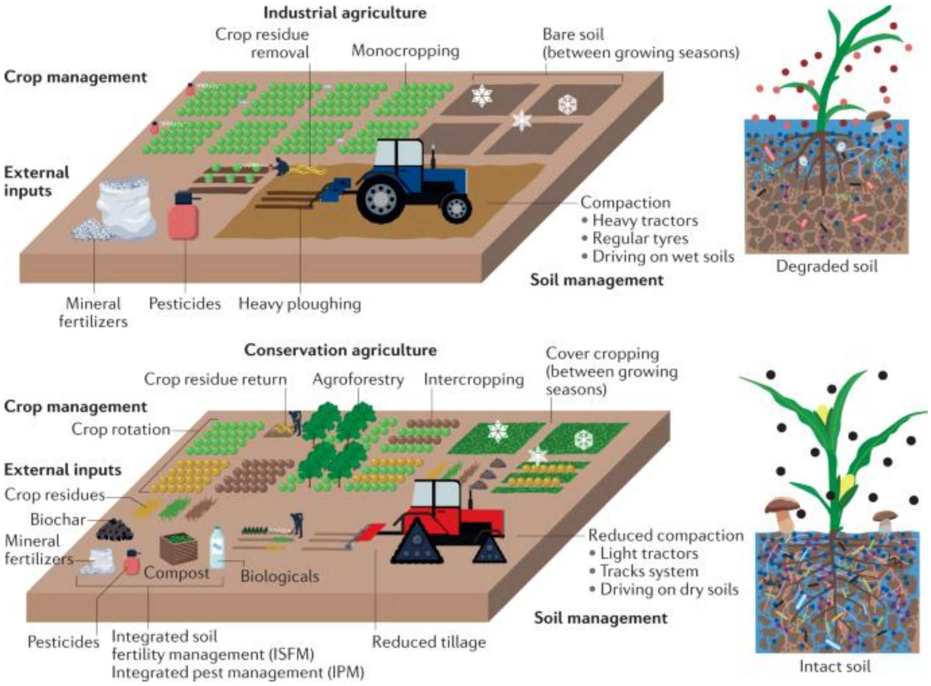

Figure 2. Different management practices. Source: "Soil structure and microbiome functions in agroecosystems". Martin Hartmann and Johan Six, Nature Reviews Earth & Environment, 2023.

Soil

One of the most important factors in the agroecosystem is the soil, and its greatest biodiversity lies in it. Soil functions have important effects on crop growth and quality, on the presence of pests and residues, on the incidence of disease, the quality of the nutrient cycle and the transfer of water, and, therefore, the entire sustainability of management [31]. Soil management is an essential component of long-term sustainable agriculture. Human use and management of land and water resources have shaped the development, persistence, decline, and regeneration of human civilizations sustained by agriculture [32]. To maintain and increase food production, conserving basic resources must become a top priority. This long-term sustainability approach tends to prioritize soil care in particular, as it recognizes its importance [15].

Soil degradation such as erosion, salinization or acidification, compaction, sealing, pollution, desertification, and, above all, loss of organic C are one of the greatest threats to agricultural production, water supply, atmospheric gas balances, and loss of biodiversity [33]. Soil is a natural formation of variable surface area, thickness, and composition, is an open system in equilibrium with other environmental components and is constantly changing. Soil is defined by the Soil Taxonomy as a three-dimensional natural body consisting of two fractions in a solid state (mineral and organic), liquids and gases, which is found on the earth's surface and is characterized by the presence of horizons that differ from each other in that they are the result of additions, losses, transfers and transformations of energy and matter. Soil is a finite resource and, at the scale of human life, essentially non-renewable. It performs many important functions, including biomass production, filtration and transformation of substances and nutrients, maintenance of a biodiversity pool, supply of raw materials, and carbon storage. It also underpins the life of terrestrial ecosystems and has direct effects on energy balance, water exchange, nutrient cycling, and ecosystem productivity. In defining soil, therefore, we must consider not only the complexity of the phenomena that characterize its genesis, the multiplicity, and variety of its components, but also its chemical-physical and biological diversity, which is strongly dependent on both the climatic and environmental conditions and how it is used.

Human activity has interfered and interferes strongly with the morphology of the land and soils through a series of actions linked to agricultural activity. Examples of this are the establishment of agricultural systems, the replacement of natural vegetation with cultivated vegetation (deforestation and planting), mechanical tillage, the installation of irrigation systems, and the use of fertilizers and pesticides that enter the nutrient cycles and modify the extent and composition of the mesofauna, microfauna, and microflora [34]. Anthropized soils subjected to recurrent mechanical tillage (most agricultural soils) are often devoid of organic horizons and characterized by a surface horizon originating from the tillage itself called the 'Ap' horizon. This rests on the part of the soil that has not been affected by tillage, but which may be affected by the type of management practised. The "Ap" horizon, which has a thickness determined by the depth of tillage, is made up of the mixing of the horizons that were once present in that thickness and is the one that has been most profoundly modified in its chemical and biological properties due to the addition of fertilizers, soil conditioners and pesticides, and the removal of nutrients [35]. The biogeochemical processes responsible for ecosystem functionality are disrupted or replaced by agricultural practices and inputs into agroecosystems [31]. For example, the amount of nitrogen that is supplied to agroecosystems is significantly greater than to natural ecosystems, and the relative magnitude of gaseous emissions and leaching fluxes is also greater. Nitrogen is the limiting element for the growth of most plants. The ammonia synthesis process helps to mitigate this limitation. However, this process uses fossil fuels at high temperatures and pressures to reduce atmospheric N_2 into ammonia (a form of nitrogen that can be used in various forms of fertilizers); therefore, although their use has increased agricultural production over the years, it has also led to losses of nitrogen into the environment through agricultural runoff and leaching and gas emissions (NO_X, N_2O and CO_2) [24,36]. Furthermore, several studies have reported that excessive use of mineral fertilizers can lead, in the long term, to loss of productivity resulting from adverse conditions such as increased competition between plants, loss of soil fertility, and nutrient depletion in soil [37,38]. Similarly, tillage also induces changes at a biochemical and biophysical level. It also modify relationships between organisms, with changes determining the dominance of species, the relative size of populations, and the diversity of communities [20]. For example, tillage can be an important stressor leading to a decrease in the inoculum potential of mycorrhizal fungi [39]. Changes in porosity and availability may exert selection pressure on nematodes and pest enemies [40]. However, the fundamental factor that determines the extent of the microbial and microfauna component is the availability of carbon and nitrogen. Tillage practices, particularly conventional ones such as plowing, promote the release and decomposition of organic matter by increasing soil biomass. The quantity, type, and location of organic matter influence various ecological communities. However, the long-term effects also depend on the amount of carbon reintroduced over the years [31]. Thus, important indicators of soil health respond to agricultural management. Soil health *is the capacity of soil to function as a living system, with ecosystem and land use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health* (FAO, 2008). Healthy soil will produce high yields in ideal conditions, but it will also have the ability to withstand extreme events and reduce losses. Therefore, soil health is crucial to increase adaptability and resilience to climate change [41]. Maintaining soil functioning often means focusing on maintaining or even increasing soil organic matter, which could be the real link between different environmental issues such as soil degradation, climate change, water quality, and food safety [42]. Increasing soil organic matter content reduces surface erosion (water and wind erosion), increases water availability, and enables a functional replenishment of the microbial pool. In addition, reducing soil evapotranspiration, allows a more rational use of water resources and a reduction in CO_2 emissions, both by reducing energy consumption (direct emissions) and by reducing the mineralization of SO in favor of its greater incorporation. All this translates into more rational management of soil fertility and offers, in a medium to long-term perspective, the possibility of modulating the use of mineral fertilizers so that greater productivity (qualitative and quantitative) can be achieved even in areas currently considered sensitive and marginal [43,44].

Soil organic matter

The set of organisms living in the soil with their organic residues to varying degrees of alteration and evolution constitutes the organic part of the soil. In very general terms, it is accepted that soil organic matter (OM) represents on average 5% by weight (12% by volume) of the soil [45]. Soil organic matter (SOM) consists of a complex and heterogeneous set of organic compounds that differ in their chemical and physical composition, functions, and dynamics, resulting from processes of hydrolysis, decomposition, and resynthesis of residues released to the soil by plants, animals, and microorganisms. It accumulates with a distribution that depends on the type of soil and its content varies from less than 1% by weight in sandy and arid soils to 1-5% in the surface horizons of cultivated soils to almost 100% in organic soils. Specifically, in Mediterranean agricultural soils the percentage of SOM is around 1.5% [46]. The difference in SO content in different soils is a function of vegetation cover, climate, geological parent material, time, and human intervention. The organic C reserves in the most common soil classes decrease in order: Histosols (390 Pg) > Inceptisols (267 Pg) > Oxisols (150 Pg) > Alfisols (136 Pg) > Aridisols (110 Pg) > Entisols (106 Pg) > Ultisols (101 Pg). SO can thus be considered as a mixture of compounds derived from plants, animals, and microorganisms, which exist in a continuum of degradative stages, starting with fresh biological residues and ending with substances with a high degree of transformation, which cannot be traced back to the biochemical starting compounds and are classically called humic substances. There are two demolition and evolution/transformation processes that organic matter can undergo: a rapid decomposition into simple inorganic substances (easily assimilated by plants) through a process known as mineralization, mainly carried out by the micro- and pedo-fauna; or a slow and complex transformation through a process known as humification into compounds with a chemical structure that is still little known today (humic substances) whose resistance to decomposition (mineralization) ensures their preservation in the soil [47].

-Decomposition and mineralization

Plant, animal, and microbial residues undergo various decomposition processes through various phases in which numerous macro- and micro-organisms play a decisive role. In general, the phase of reduction and physical amination of residues precedes the subsequent phases of enzymatic, chemical, and biochemical attack, although it is difficult to define a clear dividing line between these phases. Earthworms and other telluric animals provide the initial crushing of the residue, which then undergoes the subsequent enzymatic attack by microorganisms. The initial microbial attack is directed at the easily decomposable organic components, which are completely mineralized, i.e., transformed into inorganic substances, with the release of CO_2, NH_4^+, HPO_4^{2-}, SH^-, organic acids, and other partially oxidized intermediate compounds. The amount of C used depends on the composition of the plant residue, the nature of the microbial biomass, and numerous environmental parameters, such as O_2 and water availability, temperature, and pH. In the intermediate stages, numerous microbial species are engaged with further production of microbial biomass, loss of C, and development of CO_2. Finally, the terminal phases consist of the attack of the more refractory and resistant material, mainly by actinomycetes and fungi, the only ones capable of decomposing lignin in an aerobic environment. During these phases, there is a loss of CO_2 and recycling of the nutrients N, P, K, S, Ca, Mg, and micronutrients. It follows that as decomposition progresses, the C/N ratio of the soil decreases exponentially as nitrogen increases. The C/N ratio thus becomes an important indicator of the degree of mineralization of the SO, which will be greater the lower the value assumed by the ratio. An optimal value should be between 8 and 12. It is generally much higher in plant residues than in microbial residues [35,48].

-Humification

Simultaneously with the processes that lead to the complete mineralization of biochemical compounds, enzymatic and non-enzymatic reactions take place that result in the slow reorganization - by a preferentially oxidative pathway - of organic compounds, leading to the formation of new substances, termed humic. These may result from condensation reactions, nucleophilic addition, or neo-synthesis of new substances by the microbial community, creating a mixture of compounds with a highly variable structure and composition that cannot be ascribed to any of the known classes of organic compounds. Depending on the reactions taking place, the result is compounds that are different, undefined in structure, dark-colored, partially aromatic, largely hydrophilic, chemically complex, polyelectrolytes, and with a molecular weight ranging from a few hundred to a thousand daltons. Thus, humic substances do not have any chemical and physical characteristics that can be traced back to a specific biochemical compound and are generally characterized by a greater resistance to chemical and biological degradation than the starting compounds. As a result of the decomposition processes of animal and plant residues in the soil, two important fractions of organic matter arise the labile fraction and the stable fraction. The labile fraction is any readily degradable material derived from animal and/or plant residues and readily degradable microbial products. It represents 10 to

20% of the total organic matter and constitutes an appreciable reservoir of plant nutrients. Generally, this fraction is rapidly degraded and, if conditions are favorable, tends to disappear quickly from the soil. The stable fraction, on the other hand, essentially corresponds to humus. It has a long residence time in the soil (hundreds to thousands of years) and is intimately linked to the colloidal mineral fraction. Consequently, it plays a considerable role in the formation of the soil's aggregate state and its stability; it also contributes to water adsorption and natural soil fertility, as it releases its nutrients very slowly. All anthropic interventions that affect pedogenesis have a particular effect on the humus form since this concerns the most superficial and therefore vulnerable part of the soil. When interventions are particularly intense, they can induce a change from one form of humus to another, a change that can be reversible if the initial environmental conditions are re-established over time [35,48].

-Interactions with the mineral phase

Decomposition, mineralization, and humification processes occur in both organic and mineral horizons, but while in the former they control the turnover of C. In mineral horizons they are greatly influenced by the presence of the mineral phase, which can influence their direction, speed, and intensity. When a “fresh” plant, animal, or microbial residue meets the mineral phase, it can favor the formation of macroaggregates. Within the macroaggregate, oxidized organic products rich in carboxyl and phenolic groups can interact through ligand exchange or with the formation of cationic bridges with the reactive surfaces of the finer soil fractions (clay and silt), forming microaggregates. The interaction of organic compounds with mineral surfaces slows their degradation, preventing enzymatic catalysis, so these compounds are chemically protected from microbial attack. The formation of aggregates of different sizes favors an equal distribution between empty and full spaces within the soil, guaranteeing adequate porosity, which is indispensable for correct/optimal root development and favoring exchanges between solid, liquid, and gaseous phases of the soil (respiration of roots and living organisms, O_2/CO_2 exchanges, infiltration and percolation of water, capillary rise). Soil organic matter has a strong dynamism due to the large number of reactions it undergoes, so it is essential to establish the balance between inputs, i.e. the inputs of total organic materials (root exudates, animal manure, microbial extracellular secretions, plant post-mortal residues) and outputs, i.e. consumption and removals (mineralization, immobilization, and losses): the soil organic C balance can be considered in a very simplified way, as the difference between input and output and depends on the mean residence times (TRM) of the different fractions constituting the SO. It shows that the large quantities of organic residues contributed to the soil at the end of the growing cycle do not necessarily lead to an increase in the average C content of the soil. In addition, the addition of fresh organic residues may lead to a stimulation of the decomposition of native SO in the soil, referred to as the 'priming effect'. In other words, the addition of fresh organic material to the soil causes an increase in the activity of soil microorganisms, which also accelerates the degradation of native soil SO. The extent of the priming effect depends on various factors, including the relative amount of C in the added residue compared to the native C in the soil, and can cause an acceleration of degradation such that the soil C content is lower than before the addition [35,48].

Agroecological practices such as organic farming, conservation agriculture, climate-smart agriculture, and agroforestry could help stabilize or even increase agricultural productivity in the long run. However, research and funding are needed to adapt these technologies at local levels and to also make them suitable for small farmers.

Crops

Another key factor of the agroecosystem is the choice of cultivated plant species. At the world level, the major crops that guarantee the basic elements of nutrition are cereals and tubers. For this reason, both crops, over the years, have been the subject of numerous studies aimed at improving the quantity of production and the efficiency of their agronomic, nutritional, and phytoiatric management.

Wheat cultivation as a model experimental system

Cereals are the most abundant crops globally and among these, wheat is one of the most important sources of human calories (Figure 3).

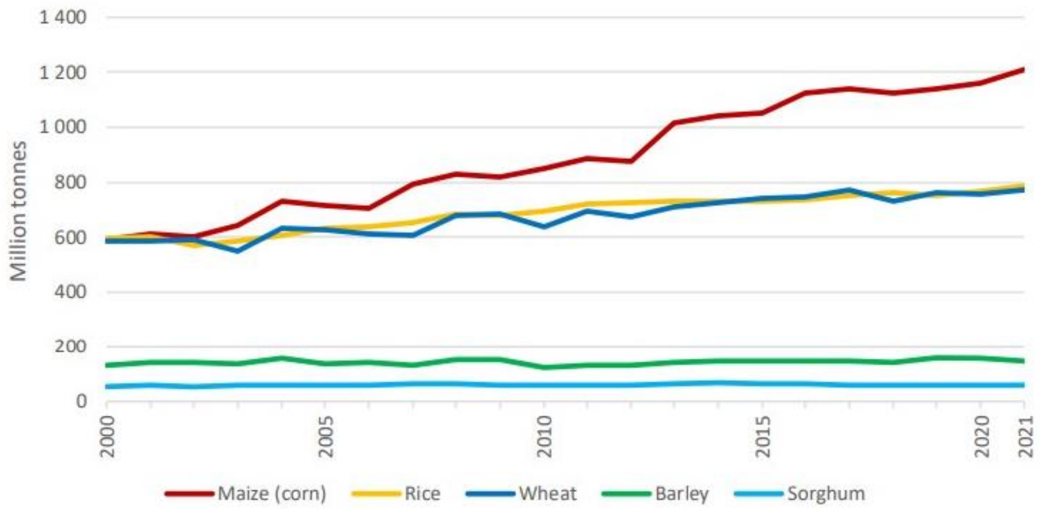

Figure 3. World production of top cereals. Source: FAOSTAT, 2022.

Wheat is the dominant crop in temperate countries. Its success depends in part on its adaptability and high yield potential, but also on the protein fraction of gluten which gives it viscoelastic properties that allow the dough to be transformed into multiple food products. Wheat also provides essential amino acids, minerals, and vitamins, as well as beneficial phytochemicals and dietary fiber components essential to the human diet. Furthermore, this crop can be easily harvested using mechanical reaper or traditional methods, and the crop can be easily stored indefinitely before consumption, provided that the water content, at the time of harvest, is less than approximately 15% of the dry weight and that parasites are controlled [49]. The first cultivation of wheat occurred about 10,000 years ago, in the Fertile Crescent, as part of the "Neolithic revolution" that saw the transition from hunting and collecting food to sedentary agriculture. These first cultivated forms were diploid wheat (*Triticum monococcum* L.) and tetraploid (*Triticum spelta* L.) [50]. Wheat belongs to six species of the genus *Triticum* belonging to the *Poaceae* family. Species with the lowest number of chromosomes, *Triticum monococcum* L., and *Triticum Urartu* Thumanjan ex Gandilyan are diploid, meaning they have two sets of chromosomes in somatic cells (2n=2x=14). The species *Triticum turgidum* L. and *Triticum timopheevi* Zhuk. are tetraploids (2n=4x=28), while the species of *Triticum* cultivated with the highest number of chromosomes are common wheat (*Triticum aestivum* L.) which is hexaploid (2n=6x=42). Among the wheat grown, *Triticum aestivum* L., or common wheat or winter or bread wheat, is the most economically important. It includes more than 20000 cultivars, belonging to the ssp. *aestivum*, which has adapted to very different environments. Its cultivation is widespread all over the world. Similarly, *turgidum* wheat, and in particular the varieties belonging to the ssp. *durum* is widely cultivated in relatively semi-arid regions such as the Mediterranean basin, China, India, and some regions with reduced rainfall in the United States, Canada, and Russia [51]. Wheat, like other autumn-winter cereals, is a long-diurnal and microthermal species. The ideal area is the temperate one (between 30° and 60° latitude), but it is also cultivated outside this range, although with more limited production. There are, in fact, various mechanisms of physiological adaptation of the crop about variations in temperature and photoperiod, which allow it to be able to synchronize the different phenological phases about periods during the year that are particularly favourable from the point of view of environmental conditions. Generally, the thermal requirements increase for the different phenological phases: 2-3°C are sufficient for germination and tillering, 10°C for stem elongation, 15°C for flowering, and 20°C for ripening. During tillering, bread wheat can withstand temperatures as low as -20°C, but subsequently, during stem elongation, a few degrees below zero can cause damage to the internodes. Generally, durum wheat is more sensitive to damage caused by low temperatures; however, modern varieties, result of genetic improvement, also have greater resistance to the cold [52]. Knowledge of genetic characteristics and particularly the growth and development pattern of wheat varieties is essential for meeting the combination of various climatic requirements for growth development and yield. The crop can be grown on a wide range of soils but prefers medium-textured ones. The optimal pH varies from 6 to 8. For a good concentration of nutrient per unit of yield, the fertilizer requirement is up to 150 kg ha^{-1} N, from 35 to 45 kg ha^{-1} P, and from 25 to 50 kg ha^{-1} K. Furthermore, the water requirement (ETm) varies from 450 to 650 mm/year depending on the climate and the length of the growing period. Grain yield and the ratio of straw to grain are linked to the duration and intensity of the water deficit, but the relationships vary depending on the growth period during which the deficits occur and on varietal differences. For most varieties and particularly for the high-producing varieties, early irrigation or heavy pre-and early-season rain can produce good yields particularly when soils are deep and have a good water-holding capacity. Only with irrigation or rain in the early growth periods plant and head number per m^2 are considered higher compared to no rain or irrigation [53]. Slight water deficits in the vegetative period may have little effect on crop development or may even somewhat hasten maturation. The flowering period is most sensitive to water deficit, this can reduce the number of heads per plant, head length, and number of grains per head. Water deficit during the yield formation period results in reduced grain weight [53]. During its 10,000 years of cultivation, selection criteria have varied over time and space, such as those for productivity. The first farmers chose plants with larger grains and more seeds per ear, subsequently, they moved on to selecting plants with high tillering, as plants with more culms and ears with many seeds. When the cultivated surface began to be a limiting factor, plants with a higher number of seeds per unit of surface area were selected. Greater importance was therefore given to genotypes that were weak competitors, that produced well in high-density crops and that responded well to fertilizers and different agronomic techniques. The success of wheat selection lies in the characteristics of the plant. It is predominantly self-pollinating and has large seeds protected by glume coatings which favor a safe establishment of the plantation and self-fertilization favors the fixation of desirable genes and recombinants deriving from crossing events. Furthermore, it responded well when moved to more fertile habitats [51]. Research in recent years has focused on its productivity, protein content, resistance to diseases and environmental stress, and the technological and nutritional quality of the grain. The genetic improvement activity has caused a gradual reduction in size, and a better distribution of dry matter between the vegetative and reproductive organs, in favor of the latter, with a higher harvest index (HI) and greater earing earliness. Nonetheless, it is still difficult to understand whether the improvements, in particular the increases in yield, are attributable to the genotype or the environment in a broad sense and in particular to the agronomic techniques as they interact with each other. Abiotic and biotic adversities also negatively influence the adaptability of cultivars to different environments, reducing their yield and decreasing the quality and healthiness of the product. Generally, most wheat diseases are caused by fungi and viruses. These pathogens can affect different organs of the plant. Among the fungal pathogens, some are obligate and highly specialized, that is, they can infect only certain genera, species, or even only certain varieties of wheat and adapt rapidly to their hosts in a process of continuous evolution. However, the improvement in disease resistance, associated with appropriate cultivation practices, has contributed considerably to increasing the productivity and quality of wheat. Proteins and starch are the main chemical components of the caryopsis that influence the nutritional and technological quality of the grain and its milling products. The quantity and quality of proteins influence many important characteristics in terms of healthiness and cost-effectiveness. Among the most important are the nutritional value of flours, the milling value, the aptitude for transformations, the color, the composition of the starch but also the toxicity of cereal-based foods for a significant fraction of consumers. Although it is strongly influenced by cultivation conditions, in particular the availability of nitrogen in the soil, the protein content is largely (40-70%) determined by the genotype and its ability to absorb nitrogen and transport nitrogenous substances into the caryopsis [54]. One of the objectives of selection programs is to increase the protein content of the caryopsis in the presence of low levels of nitrogen fertilization [51].

Potato cultivation as a model experimental system

Acting as a high-quality food with rich, complete, and balanced nutrition, containing carbohydrates, proteins, minerals, vitamins, and trace elements and having a very low-fat content and high fiber content, the potato (*Solanum tuberosum* L.), being the third largest food crop in the world, it will become one of the advantageous crops in the food security system when the cereal yield is close to the limit. In 2021, world potato production was 376 million tons, confirming itself as the most productive commodity in the tuber group (Figure 4) [55].

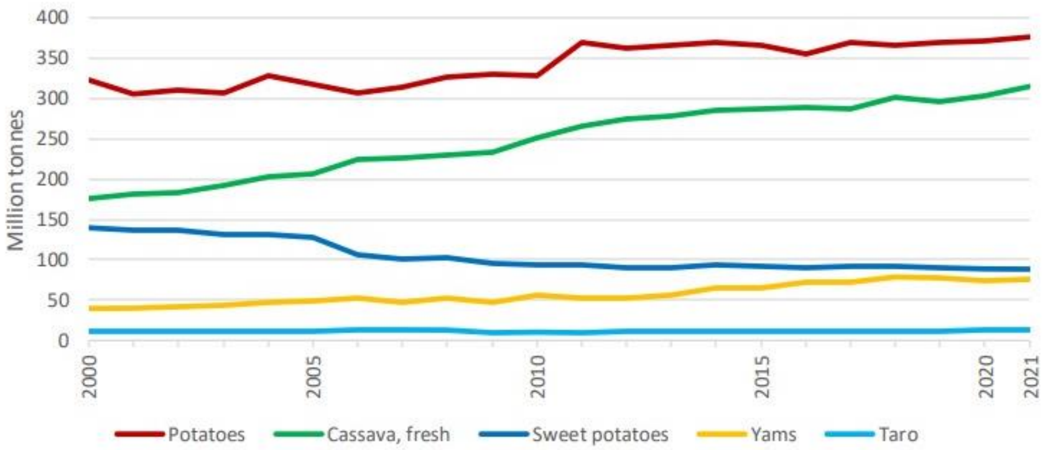

Figure 4. World production of top roots and tubers. Source: FAOSTAT, 2022.

Potato originates in the Andes from the tropical areas of high altitude. The ancient populations of the Andes Mountain range domesticated it about 7000 years ago. The crop is grown throughout the world but is of particular importance in temperate climates. Cultivated areas can be found under different environmental conditions and in regions up to 4000 meters above sea level. Its success depends on its agronomic versatility, its nutritional properties, and its multiplicity of uses. In the 1990s, global potato production grew significantly and went from being cultivated mainly in Europe, North America, and the countries of the former Soviet Union, to predominating in continents such as Asia, Africa, and Latin America [56]. The *Solanum* genus constitutes one of the most economically important taxa and includes the most variable species in terms of agronomic utility since it includes edible leafy plants (*Solanum aethiopicum* L., *S. macrocarpon* L.), edible fruit plants (*Solanum lycopersicum* L. -tomato- and *Solanum melongena* L. -aubergine-) and tuber plants (potato). The cultivated *Solanaceae* have been subjected to a long and intense selection by man, so much so that they can be considered a model for studying the evolutionary interface between plants and man. The ancient evolutionary system of the *Solanaceae*, combined with an exceptionally high level of conservation of the genome organization, make the family in general a model for the exploration of phenotypic diversity and adaptation to the natural and agronomic environment. Modern potato cultivars belong to the species *S. tuberosum* subsp. *tuberosum* (2n = 4x = 48), they share broad leaves and the ability to flower in long-day conditions. The potato has a large and rich wild germplasm which allows us to find the traits of interest necessary for the creation of a new variety such as high dry matter content, low reducing sugar content, resistance to biotic stress (fungi, insects, and nematodes) and abiotic (particularly cold and drought) and many others. Furthermore, the allelic diversity of wild species leads to an increase in allelic interactions, improving quantitative traits [57]. Potato production in Italy uses three annual harvests with cultivation from November to May (early potato), from April to September (common potato), or from August to December (leap year potato). The main share of the national product is made up of the common potato, very widespread in the northern regions, where the pedo-climatic conditions favor high yields per hectare [56]. Potato yields are influenced by temperature and the optimal average daily temperature is between 18 and 20°C. In general, a night temperature below 15°C is required for tuber induction. The optimal soil temperature for normal tuber growth is between 15 and 18°C. Tuber growth is strongly inhibited at temperatures below 10°C and above 30°C. Potato varieties can be grouped into early (90 to 120 days), medium (120 to 150 days), and late (150 to 180 days) varieties. The potato requires well-drained, well-aerated, and porous soil with a pH between 5 and 6. Fertilizer requirements are relatively high for an irrigated crop range from 80 to 120 kg ha^{-1} of N, from 50 to 80 kg ha^{-1} of P, and 125 to 160 kg ha^{-1} K. For a good concentration of nutrient per unit of yield, the crop water requirement (ETm) for a 120 to 150-day crop varies from 500 to 700 mm, depending on the climate. Good yields under irrigation of a harvest of about 120 days in temperate and subtropical climates are 25 to 35 tons ha^{-1} of fresh tubers and in tropical climates, the yields are 15 to 25 tons ha^{-1}[58]. Potato crop is susceptible to many biotic and abiotic diseases. Main abiotic constraints in potato production are zinc deficiency, salinity and high temperature and drought [59]. High nitrogen application can lower starch content, delay ripening, and make tubers more susceptible to peeling and bruising during harvest [60]. Water stress or low fertility can increase the susceptibility of the potato crop to some diseases. The most destructive diseases of the potato crop include late blight (*Phytophthora infestans*), ring rot (*Clavibacter michiganensis* ssp. *sepedonicus*), and potato Leafroll Virus which can cause total crop loss unless effective control methods are used [61]. Furthermore, in addition to vector- or soil-borne diseases, other disadvantages such as its perishability and bulkiness and its low multiplication rate challenge the creation of a functional seed system in emerging economies where institutions for rigorous management and seed quality control are weak [62,63]. Finally, actual global potato yields, with climate change, vary considerably due to temperature limitations (too cold or too hot), and limitations in precipitation or radiation [63].

Weeds

Finally, weeds are also found among the important factors of the agroecosystem. Weeds, in the past, have been defined as "those plants which are out of place, unwanted, nonuseful, often prolific and persistent, competitive, harmful, even poisonous which interfere with agricultural operation, increase labour, add to costs, reduce yields and detract from comforts of life", Crafts and Robbins, cited by [64]. Nowadays, weeds are defined as "any plant of vegetation, excluding fungi, interfering with the objectives or requirements of people" (European Weed Science Society EWRS). However, the nature of interference can also be positive. Weeds not only interact negatively with crops but can be a factor in the interaction with other organisms that have direct and indirect effects on the agroecosystem. According to an agroecological view, weeds are one of the important indicators of biodiversity. They can provide food and shelter for animal species of macro and micro fauna, can attract predatory insects beneficial for biological control, and can act as a deterrent to pathogens [65]. In addition, they can contribute to nutrient recycling, and regulate microclimate and local hydrological processes [66]. In nature, their composition is led by natural succession, which causes different communities to change in space and time. With the use of agricultural practices, this process of succession is interrupted or altered, and the weeds become specialized to the crop. It is at this time that they become an agronomic obstacle [67]. Weeds, being highly competitive, compete with crops at every stage of crop growth to take advantage of nutrients, water, light, and space. This is possible because of their ability to adapt to adverse conditions and their reproduction mechanism, which are far superior to those of crop plants [65]. The most widely used method of weed control in the world is herbicide [68]. Herbicide is a chemical used to kill or inhibit weeds. Its classification depends on the spectrum of weed control, crop, chemical type, timing and method of application, and mode of action. However, the transport of herbicides and subsequent exposure to non-target organisms have led to multiple concerns [69]. It is well known, indeed, that these chemicals not only destroy weeds but can be dispersed into the air, water, and soil; in addition, crops may have chemical residues and weeds may become resistant. Therefore, it is evident that the choice type and frequency of use therefore become essential [70]. A strategic and sustainable approach that combines different control methods to maintain weed populations at acceptable levels, while reducing the use of chemical herbicides and minimizing environmental impact is Integrated Weed Management (IWM) [71]. According to Riemens [71], this strategy is based on the assumption that it is necessary to combine multiple tactics that influence different stages of the life cycle. In addition to considering the life cycle of weeds, the tactics applied by farmers can be assigned to one of the following five pillars: diversified cropping system; choice of cultivar and establishment; field/soil management; direct control; monitoring and evaluation. Any tactic can be successful in short-term weed management but can select species that can adapt to that approach. Therefore, to achieve long-term sustainable weed control, a combination of tactics is required. The most conservative control methods include crop rotation, false seeding, high seeding density, intercropping, mulching, manual weeding, and mechanical control [72]. The goal of the sustainable approach is not the total elimination of the weed flora but to diversify the floristic composition to lower the level of competitiveness with the crop and take advantage of the ecosystem services it can produce [64].

Study context and Thesis setting

This thesis presents the activities that were carried out and the results that were obtained during the three-year period (2020-2023) of the PhD in "Plant and Animal Production Sciences", titled "Evaluation of sustainable and diverse cropping systems: crop rotation (wheat-potato) and next generation varietal selection of wheat", performed at the Department of Agricultural and Forestry Sciences (DAFNE) of the University of Tuscia. The research activity was funded by the European InnoVar Project. The experimental field tests were conducted at the "Nello Lupori" Experimental Farm of the University of Tuscia (Central Italy), under the supervision of Professor Roberto Mancinelli. The research line is focused on the evaluation of sustainable agricultural systems.

The first three chapters (Sub-study 1) are the result of a long-term experimental trial that began in 2013. In this context, I inserted myself by starting to work on data from previous years, producing various scientific publications and, at the same time, continuity of research and surveys was also given during the doctoral program, the results of which will be the subject of future evaluations. The experimental trial involves a rotation between durum wheat and potato, in which the data collected over the years has allowed us to investigate every internal and external factor of the system.

The fourth chapter (Sub-study 2) is part of the InnoVar Project, which involves the implementation of a VCU experimental trial for the phenotypic and agronomic evaluation of fifteen durum wheat varieties.

Context and Setting

Agronomic practices represent a set of techniques and strategies on which the economic, ecological, and ethical outcome of production activities depends. These include soil preparation, sowing, irrigation, fertilization, weed and pathogen control. However, over the years, agricultural development characterized by the objective of maximizing crop yields seems to have reached a limit both in terms of productivity and land use. Agricultural intensification has hurt ecosystem services, leading to a significant loss of biodiversity. Therefore, the agricultural sector must strive to apply adaptation and mitigation measures, always aiming for sustainability [73]. In the first Sub-study (Chapter 1, 2 and 3), in the choice of crops, not only was their ability to produce foods with high nutritional value considered but also their suitability within the agronomic itinerary, in which the potato represents the renewal crop and the wheat the depleting crop. The choice of rotation had the aim of reducing the phenomena of soil fatigue linked to monoculture and counteracting the development of weeds, pathogens, and harmful insects by exploiting the use of a crop that does not host them. Crop rotation is an approach that is based on the empirical observation of an improvement in the crop when it is alternated with another in the same area. This practice is particularly interesting for reducing the use of mineral fertilizers since the current crop uses the remaining nutrients and residues from previous crops [74]. The other agronomic practices taken into consideration for carrying out the test were soil tillage and fertilization. Soil tillage is mechanical intervention on the soil which has the aim of creating favorable conditions for the growth of crops. They are the main means by which the physical, chemical, and biological characteristics of the soil can be modified more or less directly. However, if the disturbance is too drastic or the soil has a high constitutional fragility, there may be irreversible degradation of the soil itself [75]. Some objectives of soil tillage are: increase in permeability and limitation of water losses through percolation and evaporation; increased softness, soft soil makes carrying out operations easier but also allows greater expansion of the roots; preparation of the seedbed, it is essential to create a suitable environment to host the seed and allow its germination; leveling of the surface; reduce weed infestation; landfilling of soil improvers or fertilizers. Despite the benefits mentioned above, conventional tillage practices have been observed to have had detrimental consequences for natural resources and environmental health [76]. The harmful effects include soil compaction as a form of degradation, mainly caused by the frequent use of heavy machinery, which leads to poor water infiltration, limited root growth and consequently losses in production; and the degradation of soil fertility due to intensive cultivation which increases the oxidation of organic matter and soil erosion [77]. Based on these constraints, it has become important to reduce the negative effects of processing as much as possible to preserve the fertility and productivity of the soil. Soil tillage is classified according to: working depth (superficial tillage < 40 cm, deep tillage > 50 cm); succession and purpose (cultivation, preparatory, complementary, and consecutive work), and work performed by the tools (tipping, splitting, mixing, mixed and special tools). To compare the effects of the different processes, we therefore chose to use a main tillage operation, plowing, and two tillage types that can be considered more conservative such as digging and subsoiling.

Plowing is a cultivation of the soil that can be more or less deep. Depending on the working depth, plowing is classified as superficial (10-20 cm), medium (20-40 cm) or deep (40-60 cm). This is a process using an overturning tool. The operation consists of detaching horizontal slices from the ground, turning them over, and crushing them to prepare the soil for sowing, planting fertilizers, and combating weeds. The soil is then processed, chopped, mixed, and aerated [78]. Thanks to the crumbling into smaller clods, the macroporosity of the soil increases, which favors root deepening and water absorption, however the excessive increase in aeration and temperature leads to an increase in the mineralization of the stable organic substance, which, in the long run, it causes a worsening of soil fertility. Furthermore, the complete overturning of the clod can bring inert soil to the surface, and its repeated use at the same depth can cause the formation of a poorly permeable surface called "plough pan" which can worsen soil drainage and nutrient absorption [79].

Digging (spading machine) is a main process, considered one of the alternative soil processes to plowing, with a depth generally ranging from 20 to 40 cm. The tool used is called a digger and is made up of a shaft in which the working parts of a reverse thrust crank mechanism are inserted. The operation consists of cutting small blocks of soil which are turned and chopped. Compared to plowing, a less accentuated inversion of the layers is obtained, with consequent less uniformity in the burial of crop residues and fertilizers and less control of weeds. However, it does not cause plough pan, allows working heavy and moist soils with the least disturbance of the soil structure, and negligible trampling [75,79].

Subsoiling is also a mechanical process that can be an alternative to plowing. Compared to the latter, it does not alter the succession of layers and does not turn the soil excessively. This operation, using a scarifying tool with curved teeth or a subsoiler with straight teeth, involves splitting the soil (cutting) without turning the layers. The depth varies from 20 to 80 cm depending on the purpose of its use. As a replacement for primary processing, it allows for greater conservation of the fertility and microbiome of the soil, avoids bringing inert or unwanted material to the surface, but also involves reduced burial of weeds and fertilizers with an accentuation of the phenomena of soil tiredness ground in the long term [75,79].

In addition to soil tillage, another management factor examined was the source of fertilization. Currently, the application of mineral fertilizers, especially nitrogen fertilizers, is widely adopted to ensure maximum crop productivity, even if their application involves costly and dangerous circumstances for the environment and human health. The massive and improper use of mineral fertilizers has caused land degradation and compromised the sustainability of many agroecosystems. This is mainly due to the large amount of fossil energy required for their production and field application, which significantly affects greenhouse gas (GHG) emissions [77,80]. Fertilization is not only aimed at maximizing production or balancing the flow of nutritional elements in the soil but also influences the quality of crops, enhancing or weakening it, with repercussions on the price and nutritional and health characteristics. The term “Fertilising product” “means a substance, mixture, micro-organism or any other material, applied or intended to be applied either on its own or mixed with other materials, to a plant or its rhizosphere with the intention of providing plants with nutrients or improving their nutritional efficiency" (EU Regulation 2019/1009). The fertilizing value of fertilizers is due to the presence of one or more chemical fertility elements, classified according to the needs of the plants into: main (Nitrogen -N-, Phosphorus -P-, Potassium -K-), secondary (Calcium -Ca-, Magnesium -Mg-, Sulfur -S-, Sodium -Na-) and microelements (Boron -B-, Manganese -Mn-, Zinc -Zn-, Copper -Cu-, Molybdenum -Mo-, Cobalt -Co- and Iron -Fe-). Based on their nature, they are distinguished into: mineral fertilizers, natural or synthetic products containing chemical elements; organic fertilizers, carbon compounds of animal or vegetable origin, chemically linked in organic form to the main elements of fertility; and organo-mineral fertilizers, obtained by reaction or mixture of one or more organic fertilizers with one or more simple or compound mineral fertilizers [81]. While mineral fertilizers are more rapidly assimilated by the plant, when the organic fertilizer needs to be mineralized first, it is known that less than half of the applied mineral nitrogen is absorbed by crops, while a part contributes to water pollution and greenhouse gas release [82,83]. The unused excess of inorganic fertilizers and the failure to return organic substances to the soil is causing a constant quantitative and qualitative degradation of the humic substances in the soil. This has caused consequences on the mechanisms of conservation, diffusion, and absorption of nutritional elements, imposing an increase in the doses of mineral fertilizers and therefore determining a negative synergism between soil degradation and the environmental load of fertilizers [78]. Developing agricultural practices that efficiently use nutrients could address these issues and achieve high crop yields with reduced impact on the environment [84]. The substitutive or mixed use of organic sources of nutrients is considered an appropriate strategy to ensure the production of safe and healthy food and improve the restoration of soil fertility while mitigating the negative effects of climate change [85]. Recently, this consolidated attention has been enriched by the effects of policies aimed at maximizing the recycling of organic waste and the economic valorization of organic by-products [86].. In addition to the advantage linked to the reuse of by-products and waste, otherwise destined for disposal, there is also a clear interest in creating alternative fertilizers to traditional ones, which are often characterized by high energy costs of synthesis or extraction and subject to import flows from countries politically unstable [86]. Recycling organic waste through a circular economy approach contributes to creating more sustainable agroecosystems. These wastes provide a large pool of mineral nutrients through a decomposition process mediated by soil microorganisms that can be effectively used for agricultural production [87].

In this first experiment, which began in 2013 and is currently underway, agricultural practices associated with traditional or conventional agriculture, such as plowing associated with mineral fertilization, were compared with more sustainable or conservative practices such as digging or subsoiling and organic fertilization. The results of this study reported in this thesis are related to production, weeds, soil fertility, and CO_2 emissions and carbon content in the soil in a durum wheat-potato rotation.

In agroecological approaches, in addition to agronomic management, crop and cultivar selection is also essential. Crop and genotype selection must first focus on agroecological suitability, which requires a thorough understanding of plant behavior to meet production and ecological standards. In particular, it is critical to study traits that confer efficiency, yield, and nutritional quality. To achieve this, the responses of crops and genotypes to various dynamics, adaptability to different soil conditions, different management, thermo-pluviometric changes, and biotic stresses must be considered. For this, the assessment must be conducted under conditions representative of the target environment, including relevant cultural practices [88,89].

It is in this context that the Sub-study (Chapter 4) as part of a European varietal selection Project (InnoVar, "Next-generation Variety testing for better cropping on European farmland"), was structured to identify, in a set of durum wheat varieties, which varieties perform best under various management and soil and climatic conditions, according to a VCU protocol. The results of this study reported in this thesis are related to production, the presence of biotic stress, and qualitative components (proteins, starch, and lipids).

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Research Aim and Objectives

Aim

Climate change represents a global threat to the agri-food sector. The aim of this thesis is to compare different agricultural practices and investigate the impact of conservative agricultural practices and assess whether they can support agri-food systems in the Mediterranean region, one of the most responsive areas to climate change.

Objectives

To achieve this aim the objectives were:

Sub-study 1: Chapter 1, 2 and 3

- To determine the variation of species composition and assess the association of weed flora in response to different soil tillage and fertilization sources. Recognize of soil tillage and fertilizer source impact on the yield of durum wheat.
- To assess the effects of soil tillage and fertilization on soil health with respect to carbon and nitrogen dynamics in potato crop, using microbial indices as indicators of mineralization–immobilization processes and as predictors of carbon sequestration and nitrogen availability in a longer period.
- To study the effects of soil tillage and fertilization on CO_2 emissions, soil organic C content, soil temperature and volumetric water content in a two-year durum wheat-potato rotation system.

Sub-study 2: Chapter 4

- To evaluate the performance of varieties of durum wheat and their characters under different management approaches relating to fungicides and growth regulators (PGR), organic regime and irrigation.

Chapter 1

Weed Spectrum in Durum Wheat under Different Soil Tillage and Fertilizer Application in Mediterranean Environment

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Verdiana Petroselli[1], Emanuele Radicetti[2]*, Alireza Safahani Langeroodi[3], Mohamed Allam [1] and Roberto Mancinelli[1]

[1] Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, Via San Camillo de Lellis, snc, 01100 Viterbo, Italy; verdiana.petroselli@unitus.it (V.P.); mohamed.allam@studenti.unitus.it (M.A.); mancinel@unitus.it (R.M.)

[2] Department of Chemical, Pharmaceutical and Agricultural Sciences (DOCPAS), University of Ferrara, Via Luigi Borsari, 46, 44121 Ferrara, Italy

[3] Department of Agronomy, Payane Noor University, Tehran 845683111, Iran; safahani.ali@gmail.com

***** Correspondence: emanuele.radicetti@unife.it

Abstract

Agricultural intensification may cause significant changes in weed density due to high weed competitiveness. Therefore, sustainable practices are to be designed to get maximum benefits of plant biodiversity in the agroecosystems. Field experiments were conducted in 2013/2014 and 2014/2015 to evaluate the impact of fertilizer source and soil tillage on weed spectrum in durum wheat (*Triticum durum* Desf.). Treatments in this study were: (I) two fertilizer sources (mineral fertilizer -MIN- and municipal organic waste -MOW-), and (II) three tillage regimes (plowing -Plo-, subsoiling -Sub- and spading -SM-). A randomized complete block design with three replications was adopted. Data on weed density and biomass were collected at the wheat tillering stage. Weed density was higher in MOW than MIN (53.8 vs. 44.0 plants·m^{-2}), especially in 2014/2015, while S was the highest among tillage regimes (58.2 plants·m^{-2}). Annual and monocots species were always the highest in subsoiling (43.5 and 10.1 plants·m^{-2}). The density of perennial and dicots species was higher in MOW compared with MIN plots, regardless of soil tillage management. Weed community, in terms of weed species composition, varied between the two fertilizer sources, while among soil tillage regimes, it only differed between plowing and subsoiling. Based on the analysis of weed community composition, annual dicot species were mainly associated with plowing, while monocots tended to be associated with MIN fertilizer. Spading tillage may be a useful strategy for managing weed diversity under organic fertilization, where mineral soil nitrogen availability was limited. Conversely, the spading machine produced lower grain yields than plowing with mineral fertilizer application.

Keywords: sustainable crop management; biodiversity; weed association; weed community composition; crop yield.

1. Introduction

Conventional agriculture, based on intensive soil tillage and excessive mineral fertilizer applications, aims only to maximize crop yield. These practices are considered unsustainable because they weaken the resilience of agroecosystems and thus cause a considerable loss of the structural integrity of the system, in terms of reduced capacity to provide a wide range of ecosystem services, not only for food production but also linked to other such as provisioning, regulating, and supporting services that contribute to maintaining the agroecosystems as sustainable [1]. However, the soil tillage practice offers several advantages for crop cultivation, mostly through increased aeration and softness. On the other hand, it is also one of the main causes of soil compaction, especially when using heavy machinery, such as plowing vehicles [2]. In addition, the use of such machinery requires more fossil fuels and causes more greenhouse gas emissions [3]. Moreover, the use of moldboard plow tends to bury the organic matter (SOM) in the anoxic deep layer of soil instead of allowing it to be processed in the active zone of soil micro-fauna and micro-organisms [4]. Jaskulska *et al.* [4] highlight the need to limit the unfavorable effects of conventional agriculture on the environment, replacing it with alternative approaches to crop cultivation, such as conservation agriculture.

Conservation agricultural practices mainly based on minimum disturbance of the soil (minimum tillage or no tillage) and the diversification of plant species improve biodiversity and the natural biological processes and contribute to greater water and nutrient use efficiency, supporting plant production [5]. Moreover, conservation tillage practices promote favorable changes in soil properties, preserve soil moisture, especially during the precipitation deficit, and increase the content of SOM in the 0–20 cm soil layer, as previously observed by Jaskulska and Jaskulski [6]. Therefore, alternative soil tillage to conventional plowing, such as spading and subsoiling, appear to be more suitable, mostly because they allow a decrease in external inputs, in terms of reduced fuel consumption, and result in similar crop yield compared with conventional tillage practices, even if their suitability is carefully evaluated based on the different climate, soil and crop conditions [7].

Mineral fertilizers provide a high amount of nutrients that are used for improving crop yield; however, their overuse and repeated applications affect the stability of agroecosystems and their living communities, including weeds [8,9]. Several studies reported that excessive use of mineral fertilizers may lead to the loss of productivity resulted from adverse conditions for crop growth, i.e., high competition for limited resources, loss of fertility, depletion of soil nutrients and environmental pollution [10,11]. Accordingly, the high percentage of nitrogenous fertilizers may cause pollution of surface water and groundwater [12]. In addition, mineral fertilizers, especially nitrogen, may affect certain weeds by breaking their dormancy and thus affect weed species composition, thus, limiting their availability for the crops [13]. Considering that many weeds are high consumers of nitrogen, the application of organic fertilizers, characterized by slow release, reduce the availability of soil nitrogen and, therefore, affect weed demographic processes and crop–weed competitive interactions [13–15]. Among the organic fertilizers, municipal organic waste (MOW) could represent a low-cost alternative nutrient management that may provide several benefits for promoting and enhancing agroecosystem health. Indeed, the MOW is mainly composed of properly composted kitchen and yard waste and is used as a way of diverting organic waste materials from landfills while creating a product, at relatively low cost, that is suitable for agricultural purposes. The MOW use in agriculture is growing as soil conditioner and fertilizers to meet crop nitrogen requirements and for increasing the organic matter in the soil. Moreover, by increasing organic C content in the soil, MOW application impacts soil parameters, directly and indirectly. Other benefits include improving soil structure and aggregate stability and promoting more balanced microbial biomass that will act over time as a reserve of mineral elements by retaining them in the surface horizons and gradually releasing to plants [16,17]. Although the adoption of MOW is recommended for the potential benefits in agriculture, its safe use is related to the quality of the materials and metals and salt contents that can generate problems in case of overuse [18].

Durum wheat represents one of the major crops cultivated in the Mediterranean basin, especially in Italy, with notable relevance from an economical and cultural point of view where it is considered a staple crop for many people across the world [19]. When durum wheat is grown in a Mediterranean environment, it often suffers from a soil water deficit in spring, where a low rainfall during the flowering stage causes strong intraspecific competition among wheat plants and weeds, which could noticeably reduce the potential yield of the cereal [20]. Considering that durum wheat is a weak competitor [21,22], it is of great importance to evaluate the weed community composition triggered by management practices, such as soil tillage and fertilization sources, in order to avoid highly competitive weed species that could decrease the yield capacity of the cereal crops [23]. Indeed, according to the findings of Usman *et al.* [24], weeds represent the major concern on wheat-based rotations, and conventional tillage practices deteriorate soil and water quality, resulting in serious concerns about the future potential for productivity growth and long-term sustainability of wheat cultivation. In addition, the application of high levels of mineral nitrogen fertilizers stimulates the growth of weeds more than the growth of crops, resulting in enhanced competitiveness against cultivated plants [13]. Therefore, alternative management practices are needed to maximize crop productivity while minimizing potential negative impacts of weeds [25,26].

From an agroecological point of view, the weeds are an important component of the biodiversity [27]. Their ecological role within the system is to support more diversified biota. They can be beneficial to the system by contributing to the recycling of nutrients, increasing the presence of micro-fauna, and regulating the microclimate and local hydrological processes. Furthermore, weed diversity has been suggested to decrease the likelihood of developing herbicide resistant weed communities and provide resources for beneficial organisms and enhance soil quality [28]. Weeds can also impair the performance of the agroecosystem, entering strong competition with the crop or becoming a vector of pathogens or insect pests [29]. The optimal weed diversity will be the one without truly dominant species, combined with a low overall density [30]. Despite the importance from an agroecological point of view, it is important to keep weed infestation below the harmfulness threshold to avoid severe economic losses by means of soil tillage, fertilization source and herbicide application as a part of an integrated weed management strategy.

Worldwide, wheat cultivation is generally based on intensive soil tillage operations supported by the high rate of nitrogen fertilizers that are detrimental to the agroecosystem’s sustainability, also including the loss of biodiversity as weed species composition. Although there are many studies that reported the effect of intensive practices on the weed spectrum, to date, few studies have reported the interaction effects of soil tillage and fertilization sources on weed flora composition. Indeed, the adoption of some alternative agronomical strategies should be adequately evaluated both agronomically and environmentally. A better understanding of these issues could contribute to more effective use of agronomic practices to manage weeds and to support sustainable management practices in the Mediterranean agroecosystems. Therefore, this study hypothesized that spading and sub-soiling and organic fertilizers can contribute to maintaining weed diversity in durum wheat cultivation, compared with plowing and mineral fertilizers. The main objectives of this study are: (I) to determine the variation of species composition of weed flora in response to different soil tillage and fertilization sources; (II) to assess the association of weed flora to different soil tillage and fertilization sources; and (III) recognition of soil tillage and fertilizer source impact on the yield of durum wheat.

2. Materials and Methods

2.1 Site Description

Field experiments were carried out in two consecutive growing seasons (2013/2014 and 2014/2015) of durum wheat at the research farm of the University of Tuscia located in Viterbo, Latium region (lat. 42°42', long. 12°07' and alt. 310 m a.s.l.). In both growing seasons, trials were performed in Entisol soil with sandy-loam texture (USDA) classified as a *Typic Xerofluvent* of volcanic origin with the following particle size distribution in the first 30 cm of soil layer: 760 g kg^{-1} of dry sand, 130 g kg^{-1} of dry silt, 110 g kg^{-1} of dry clay (loamy sand). Moreover, the soil was characterized by 0.97% of organic matter content, 0.15% total nitrogen and 6.9 pH (H_2O). The experimental area is typical of a Mediterranean environment with a mean temperature of 14 °C and a yearly rainfall of 780 mm (average of the last 30 years period).

2.2 Experimental Details

The study was conducted with the aim of evaluating the spectrum of weed flora in durum wheat subjected to different tillage regimes and fertilizer sources. The field trials were conducted in fields cultivated with potato (*Solanum tuberosum* L.) in the previous year as a crop rotation commonly adopted by the local farmers. The field experiment was arranged in a randomized complete block design with a factorial arrangement of treatments and three replications of each treatment. The experiment included (I) three tillage regimes (conventional tillage performed with moldboard plow (hereafter called Plo), alternative soil tillage performed with subsoiler (hereafter called Sub), and another alternative soil tillage performed with a spading machine (hereafter called SM)), and (II) two fertilization sources (mineral based on the application of mineral fertilizers (hereafter called MIN) and organic based on the application of municipal organic waste (hereafter called MOW)). The field experiment consists of 18 plots (3 soil tillage × 2 fertilizer sources × 3 replications, Figure 1). Each plot had a size of 60 m^2 (6 × 10 m). Furthermore, 5-m-wide alleys separated all plots of the experimental field to carry out all farming operations.

Figure 1. Experimental field plan in the 2013/2014 growing season of durum wheat. MIN = mineral fertilization; MOW = municipal organic waste; Plo = plowing; Sub = subsoiling; SM = spading machine.

2.3 Weather Conditions during Durum Wheat Cultivation

The durum wheat growing season lasted for 218 and 213 days in 2013/2014 and 2014/2015 growing seasons, respectively. The total rainfall was 822 mm in 2013/2014, uniformly distributed during the whole growing season of durum wheat, except two peaks of 157 and 136 mm in January and June, respectively (Figure 2). The cumulated precipitation was low (529 mm) in the 2014/2015 growing season, mainly concentrated from November to February, while it was limited in the following period. Air temperatures follow the long-term average trend and gradually decrease after durum wheat sowing until February, and then air temperature gradually increased, reaching the highest temperature before the wheat seed harvesting. The average air temperature was similar between the growing seasons (12.1 °C), minimum temperatures were a bit hotter in 2014/2015 compared with 2013/2014 (on average 7.4 vs. 6.9 °C). In both growing seasons, minimum temperatures seldom dropped to 0 °C in winter, except in January 2014 when air temperature dropped to 0 °C two times.

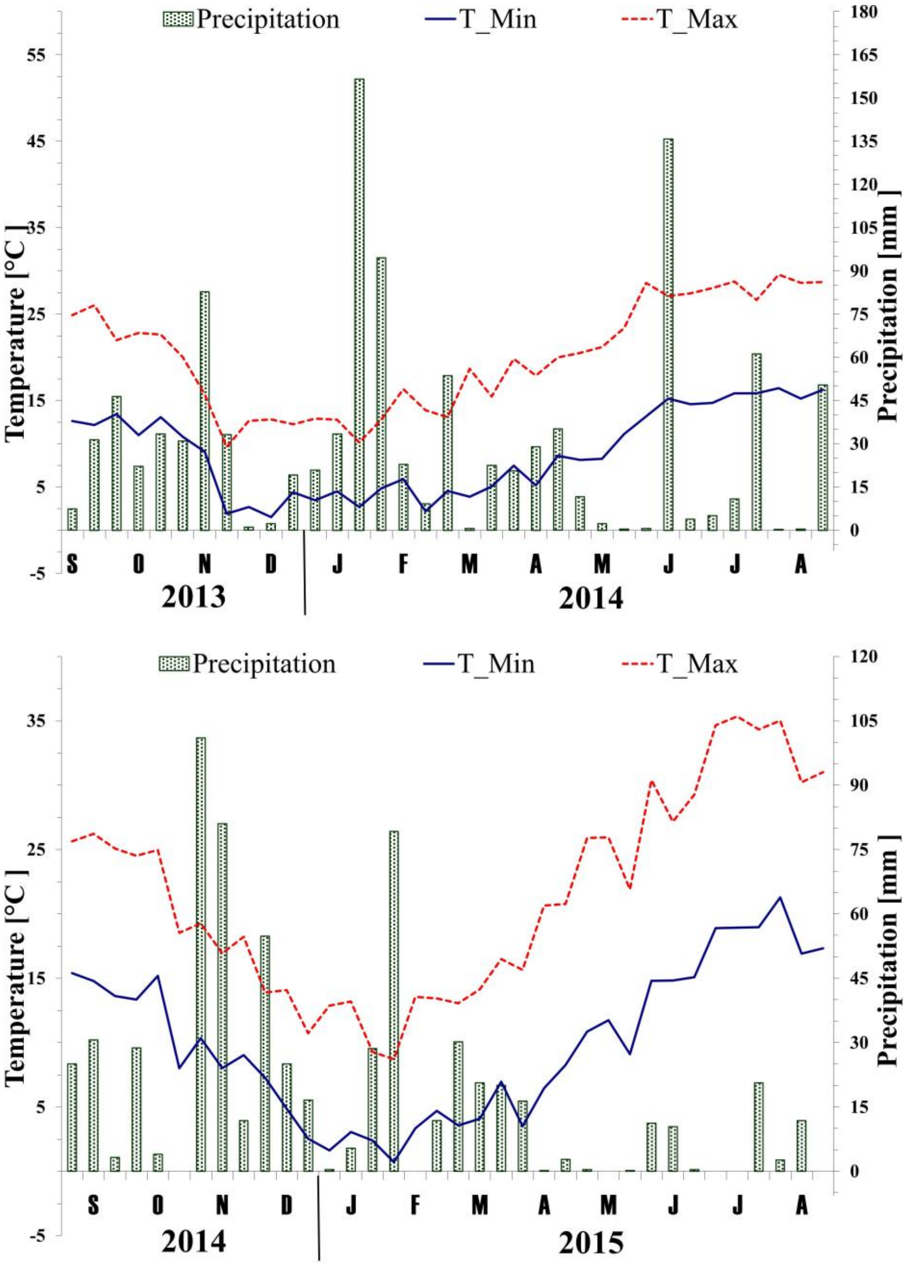


Figure 2. Weather data at 10-day intervals (maximum (red line), minimum (blue line) temperatures (°C) and rainfall (columns, mm)) at the experimental site during the experimental period in 2013/2014 and 2014/2015 growing seasons of durum wheat.

2.4 Crop Management

The seedbed of durum wheat was prepared in September of each growing season according to the selected soil tillage treatments. All main tillage (Plo, Sub and SM) were done up to 30 cm of the soil layer and then followed by a disk harrowing up to 10 cm of soil depth performed twice until durum wheat sowing. The machines used in Plo, Sub and SM treatments were Angeloni APS M35 [31], Dondi RHP-5M [32] and Tortella 005-100 [33], respectively. In mineral fertilizer source, fertilization practices were performed according to the local practices by applying the total amount of 80 kg of P_2O_5 ha^{-1} as a triple superphosphate applied before the last disk harrowing for seedbed preparation and a total of 120 kg of N ha^{-1} divided into three rates according to the common practices adopted by the farmers of the area: the first N application (30 kg of N ha^{-1} as a calcium nitrate) was carried out at the beginning of the tillering stage in January; the second N application (40 kg of N ha^{-1} as ammonium nitrate) was distributed at the end of the tillering stage in February; and the last N application (50 kg of N ha^{-1} as urea) was applied at the beginning of stem elongation in March. In the organic fertilizer, municipal organic waste was applied at the rate of 10,000 kg ha^{-1} to apply the same amount of nitrogen applied in the mineral fertilizer treatments. The rate of organic fertilizer application was calculated considering the theoretical mineralization of MOW considering the demand of 120 kg of N ha^{-1} as common practices adopted by the farmers. The characteristics of the MOW fertilizer are reported in Table 1. The chemical analysis of MOW showed that all heavy metals are widely below the legal limits (Table 1). The MOW fertilizer was applied before the last disk harrowing for the seedbed preparation.

Table 1. Chemical and physical characteristics of municipal organic waste (MOW) adopted in the experiments and admitted limits based on D.lgs 75/2010.

Parameter	Methodology	Values	Limit
Moisture (%)	DM 21/12/2000 GU n. 21 26/01/2001	19.4	<50
pH	DM 17/06/2002 GU n. 220 del 19/09/2002 suppl.7 Met III.4	7.8	6–8.8
Conductivity (dS m^{-1})	DM 17/06/2002 GU n. 220 del 19/09/2002 suppl.7 Met III.4	3.82	-
Organic carbon (%)	DM 13/09/1999 SO n. 185 GU n. 248 21/10/1999 Met. VII.1	25.7	>20
Organic nitrogen (%)	DM 13/09/1999 SO n. 185 GU n. 248 21/10/1999 Met. VII.1	2.37	-
C/N ratio	DM 13/09/1999 SO n. 185 GU n. 248 21/10/1999 Met. VII.1	10.7	<25
Phosphorous	EPA 3052 1996 + EPA 6010D2018	0.5	-
Cadmium (mg kg^{-1} DM)	EPA 3052 1996 + EPA 6010D2018	<1.0	<1.5
Copper (mg kg^{-1} DM)	EPA 3052 1996 + EPA 6010D2018	70.1	<230
Mercury (mg kg^{-1} DM)	EPA 7473 2007	0.10	<1.50
Nickel (mg kg^{-1} DM)	EPA 3052 1996 + EPA 6010D2018	16.8	<100
Lead (mg kg^{-1} DM)	EPA 3052 1996 + EPA 6010D2018	34.5	<140
Zinc (mg kg^{-1} DM)	EPA 3052 1996 + EPA 6010D2018	146.0	<500
Chrome VI (mg kg^{-1} DM)	DM 08/05/2003 Suppl.8 GU n. 116 21/05/03	<0.10	<0.5

Durum wheat, cv. “Claudio”, was sown on the same day in all treatments on 27th November 2013 and 25th November 2014 by means of experimental planter (Plotseed TC—Wintersteiger AG, Ried im Innkreis, Austria) at the seed density of 450 seeds m^{-2} with row distance of 12.5 cm and about 3 cm of depth. Weeds were managed by means of herbicide (Mesosulfuron-Metile 3% + Iodosulfuron. Metil-Sodium 3% + Mefenpir-Dietile 9%) applied at the end of wheat tillering in all treatments, as commonly applied by the farmers of the area. Durum wheat was harvested at physiological maturity of kernels on 3 July 2014 and 26 June 2015.

2.5 Observations

Weed biomass and weed density (total and specific) were taken the day before herbicide application in all plots. A sampling area of 0.25 m^2 (0.5 × 0.5 m) was placed randomly four times, for a total of 1 m^2, in a uniform area inside each plot to record weed diversity and density. Weeds were uprooted manually and then divided per species in order to determine weed species composition and specific weed species density. Total weed density was obtained by adding the specific densities of all species, while the total weed biomass was evaluated by weighing the whole sample from each plot after oven-dried at 80 °C until constant weight. At durum wheat physiological maturity, the whole aboveground biomass from four 2-m long adjacent rows (1 m^2), harvested in the center of each plot, was collected by hand clipping the wheat plant at ground level and analyzed for evaluating yield and yield components.

2.6 Data Analysis

Statistical analysis was carried out by performing analysis of variance (ANOVA) among the observed data. The variance analysis of field data was carried out using the software JMP v. 4.0 [34] for the 2-year period applying a randomized complete block design with three replications. A Bartlett test was performed to test if data have equal variance [35]. Weed density data were square root transformed (x + 0.5) before analysis in order to homogenize the variance (Gomez and Gomez, 1984). Data reported in the tables are back transformed. A two-way factorial experimental design was performed for weed and wheat characteristics where soil tillage and fertilization source were the treatments, and the growing season was considered as the repeated measure. Fisher's protected least significant differences (LSD) at the probability of 0.05 ($p \leq 0.05$) was adopted to compare the main and interaction effects.

The multi-response permutation procedure (MRPP) was applied to test whether there was a significant difference between weed species composition among soil tillage and fertilizer source groups, as suggested by McCune and Grace [36]. The MRPP provides a T-statistic that describes the separation among groups (the more negative T is, the stronger the separation is) and its associate significance. The MRPP analysis was carried out using the BLOSSOM software [37]. Moreover, the canonical discriminant analysis (CDA) was applied to identify the associations between each weed species with the soil tillage and fertilizer source. The result of CDA analysis was reported in a two-dimensional canonical discriminant structure plot, where the appearance of weed species and treatments in the same ordination space indicates association between them. The size of the standardized discriminant function coefficient associated with each variable indicates the contribution of that weed species to the discriminant function (soil tillage or fertilizer source).

3. Results

3.1 Weed Characteristics in Durum Wheat

There was an interaction between soil tillage regimes and fertilization sources for weed density and aboveground biomass during both growing seasons of durum wheat (Supplementary Table S1). Weed density observed in the field study ranged from 67.3 to 34.3 plants·m^{-2}, and it was higher in 2013/2014 compared with the 2014/2015 growing season (on average 51.3 vs. 46.5 plants·m^{-2}, respectively). Moreover, weed density was greater in MOW than the MIN fertilization source (on average 53.8 vs. 44.0 plants·m^{-2}, respectively). Differences were evident among all soil tillage treatments in 2014/2015 and only under Sub treatment in 2013/2014 (Table 2). The weed density observed in Sub tillage was the highest regardless of the fertilization (on average 58.2 plants·m^{-2}) and was intermediate in SM and low in Plo under the MOW fertilization source, while similar values were detected between Plo and SM under the MIN fertilization source in both growing seasons (Table 2). Weed aboveground biomass was the highest in Sub-MOW 2014/2015 (94.0 g· m^{-2} of DM) and the lowest in Plo-MIN in 2014/2015 (42.5 g· m^{-2} of DM) and showed a similar trend to that described for weed density. Furthermore, it was higher in 2013/2014 than 2014/2015 (66.2 vs. 63.0 g· m^{-2} of DM, respectively). Although the weed biomass was higher in MOW compared with MIN fertilization source (on average 72.4 vs. 56.8 g· m^{-2} of DM, respectively), the main differences between fertilizer sources were observed in the 2014/2015 growing season regardless of the soil tillage practice. Inversely, in 2013/2014, growing season weed biomass was higher in MOW than MIN only under SM tillage, while similar values were observed under Sub and Plo soil tillage systems (Table 2). Generally, in both growing seasons and fertilizer sources, weed biomass was the highest under S tillage followed by SM than Plo tillage (on average 77.4, 61.1 and 55.3 g· m^{-2} of DM, respectively), except in 2013/2014-MOW where the weed biomass was higher under Sub and similar between Plo and SM tillage regimes (Table 2). In both growing seasons of durum wheat, weed flora composition was mainly composed of annual species compared with perennial ones (on average 41.0 vs. 7.9 plants· m^{-2}, respectively, Table 3). The data analysis showed that the number of annual weed species was affected only by soil tillage treatments ($p < 0.05$, Supplementary Table S1). In fact, annual weeds were similar among both fertilizer sources, and they were high in Sub, intermediate in SM and low in Plo tillage (43.5, 40.0, and 39.4 plants· m^{-2}, respectively). Perennial weed species were affected by soil tillage × fertilization source (Plo < 0.05, Supplementary Table S1) and tended to be higher in MOW than MIN fertilizer sources (on average 8.9 vs. 6.9 plants· m^{-2}, respectively), even if the main differences were observed under Sub tillage (Table 3). In both fertilization source treatments, the perennial weed species were the highest in Sub followed by SM and Plo tillage (on average 14.7, 6.8, and 2.2 plants· m^{-2}, respectively).

Table 2. The interaction effect of year × fertilization source × soil tillage on the weed density and weed aboveground biomass in durum wheat. Values followed by the same lower-case letters in columns are not different according to LSD (0.05). Values followed by the same upper-case letters in rows (within year) are not different according to LSD (0.05). MIN = mineral fertilization; MOW = municipal organic waste; Plo = plowing; Sub = subsoiling; SM = spading machine. LSD = least significant differences.

	Weed Density (plants· m^{-2})				**Weed Aboveground Biomass (g· m^{-2})**			
	2013/2014		**2014/2015**		**2013/2014**		**2014/2015**	
	MIN	**MOW**	**MIN**	**MOW**	**MIN**	**MOW**	**MIN**	**MOW**
Plo	44.3 ^{bA}	46.0 ^{bA}	34.3 ^{bB}	42.0 ^{cA}	54.1 ^{bA}	55.0 ^{cA}	42.5 ^{cB}	69.6 ^{cA}
Sub	56.3 ^{aB}	63.7 ^{aA}	45.3 ^{aB}	67.3 ^{aA}	75.6 ^{aA}	77.0 ^{aA}	62.8 ^{aB}	94.0 ^{aA}
SM	47.0 ^{bA}	50.3 ^{bA}	36.7 ^{bB}	53.3 ^{bA}	54.3 ^{bB}	61.9 ^{bA}	51.2 ^{bB}	77.0 ^{bA}
LSD	7.21				6.34			

Table 3. The main effect of soil tillage on annual and monocot weeds and the interaction effect of fertilization source x soil tillage on perennial and dicot weeds in durum wheat. Values followed by the same lower-case letters in columns are not different according to LSD (0.05). Values followed by the same upper-case letters in rows (within fertilization source) are not different according to LSD (0.05). MIN = mineral fertilization; MOW = municipal organic waste; Plo = plowing; Sub = subsoiling; SM = spading machine; LSD = least significant differences.

	Weed Density Characteristics (plants· m^{-2})							
	Annual		**Perennial**		**Monocots**		**Dicots**	
	MIN	**MOW**	**MIN**	**MOW**	**MIN**	**MOW**	**MIN**	**MOW**
Plo	37.0 ^{aB}	41.8 ^{bA}	2.3 ^{cA}	2.1 ^{cA}	3.7 ^{aB}	9.3 ^{bA}	30.0 ^{bB}	40.3 ^{cA}
Sub	38.7 ^{aB}	48.3 ^{aA}	12.2 ^{aB}	17.2 ^{aA}	6.8 ^{aB}	13.3 ^{aA}	37.5 ^{aB}	58.7 ^{aA}
SM	38.7 ^{aB}	44.3 ^{abA}	6.1 ^{bA}	7.5 ^{bA}	4.5 ^{aB}	13.3 ^{aA}	29.5 ^{bB}	47.3 ^{bA}
LSD	5.94		1.42		3.78		2.81	

The density of dicots weed species was higher compared to monocots species (on average 40.6 vs. 8.7 plants·m^{-2}, respectively). The dicots weed species were affected by soil tillage x fertilization source interaction ($p < 0.05$, Supplementary Table S1). The dicot weed species were higher in 2013/2014 than 2014/2015 growing seasons (41.8 vs. 39.3 plants·m^{-2}, respectively). Dicot weed density was greater in MOW than MIN fertilizer sources (on average 48.8 vs. 32.3 plants·m^{-2}, respectively); moreover, they tended to be the highest under Sub tillage, intermediate in SM and low in Plo tillage (on average 48.1, 38.4, and 35.2 plants·m^{-2}, respectively), but under MIN fertilizer sources, SM and Plo tillage showed similar values to dicots weed species (Table 3). The monocots species were affected by only the soil tillage treatments ($p < 0.05$) and their density was higher and similar under both Sub and SM tillage (on average 9.8 plants·m^{-2}) compared with Plo tillage (6.5 plants·m^{-2}).

The results of the MRPP analysis suggested that in both growing seasons of durum wheat, the MIN and MOW fertilization source groups were characterized by different vegetation assemblages; however, the magnitude of the differences (*T* value) in 2013/2014 was higher than 2014/2015 (-5.73 vs. -3.61, respectively, Table 4). The canonical discriminant analysis on the weed species observed at the end of durum wheat tillering was in accordance with the MRPP analysis, and it showed that the first two canonical variables explained 67% of the total variance for fertilizer source treatments (Figure 3A). Indeed, the CDA analysis showed that monocot weed species such as *Lolium perenne*, *Avena sterilis*, *Phalaris minor*, *Phalaris canariensis* were associated with MIN fertilizer sources together with *Galium aparine* and *Convolvulus arvensis*. Conversely, the vectors of most of the dicots weed species were located in the same orientation space of MOW fertilizer sources (Figure 3A).

Table 4. Test statistic from the multi-response permutation procedure (MRPP) for multiple paired comparisons to evaluate the main effects of fertilization source and soil tillage on the density of each weed species that determined the floristic composition in 2013/2014 and 2014/2015 observed in durum wheat. P is the probability of significant differences among selected groups. T is the T-statistic. MIN = mineral fertilization; MOW = municipal organic waste; Plo = plowing; Sub = subsoiling; SM = spading machine

	2013/2014		**2014/2015**	
	T	***p***	**T**	***p***
Fertilization				
MIN vs. MOW	−5.73	<0.0001	−3.61	0.0041
Soil tillage				
Plo vs. SM	−0.58	0.2368	−0.09	0.5134
Plo vs. Sub	−2.63	0.0225	−3.91	0.0029
SM vs. Sub	−0.26	0.3468	−1.43	0.0883

The results of the Multi-Response Permutation Procedure (MRPP) showed that the T-statistic for defined groups of soil tillage were negative and significant when plow (Plo) was compared with subsoiler (Sub) in both growing seasons, while no significant differences were observed between P vs. spading machine (SM) and SM vs. Sub multiple paired comparisons (Table 3). The CDA on the weed species observed at the end of durum wheat tillering was in accordance with the MRPP analysis, and it showed that the first two canonical variables explained 73% of the total variance for soil tillage treatments (Figure 3B). Based on the tillage treatments, *Lolium perenne*, *Sonchus arvensis*, *Papaver rhoeas*, *Matricaria camomilla*, *Fumaria officinalis*, *Fallopia convolvulus*, *Rapistrum rugosum*, *Chrysantemum segetum*, *Avena sterilis*, *Cirsium arvensis* and *Galium aparine* were associated with subsoiler tillage, while *Anagallis arvensis*, *Centaurea cyanus*, *Phalaris canariensis* and *Sylibum marianum* were associated with spading soil tillage and *Capsella bursa-pastoris*, *Sinapis arvensis*, *Stellaria media* and *Veronica hederifolia* were associated with plough soil tillage (Figure 3B).

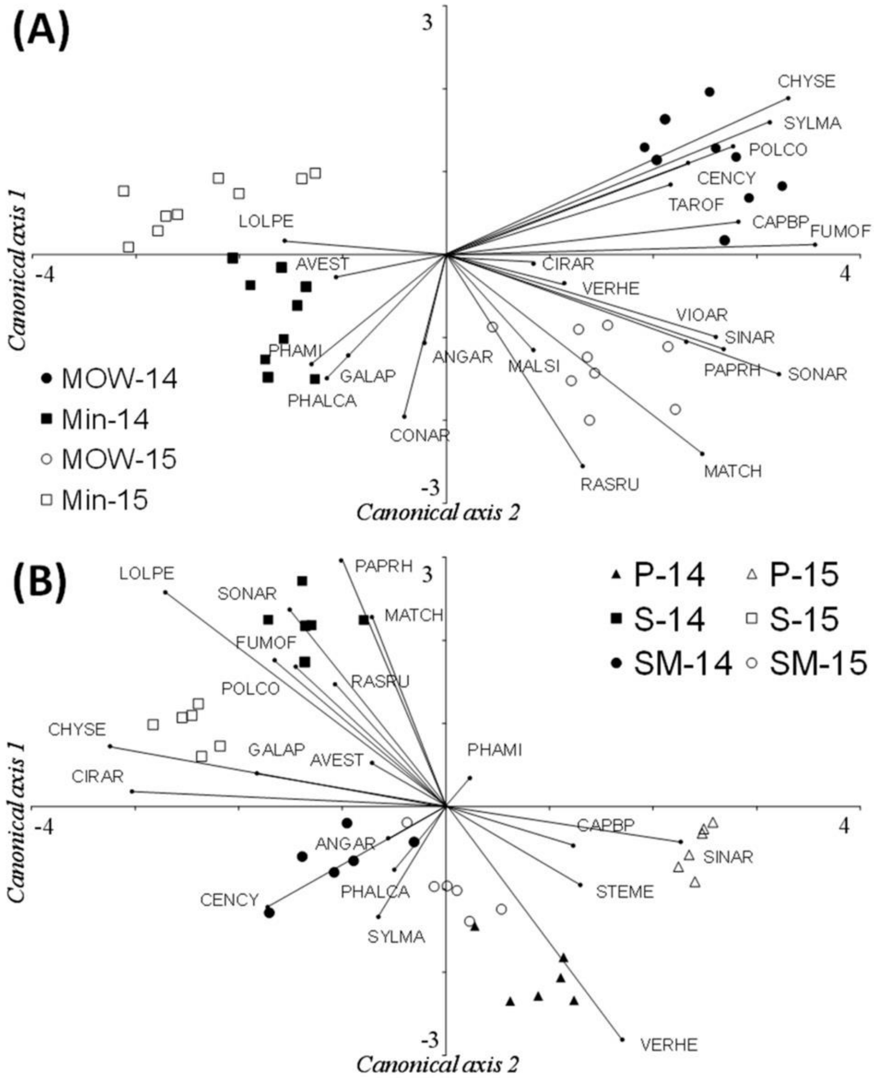
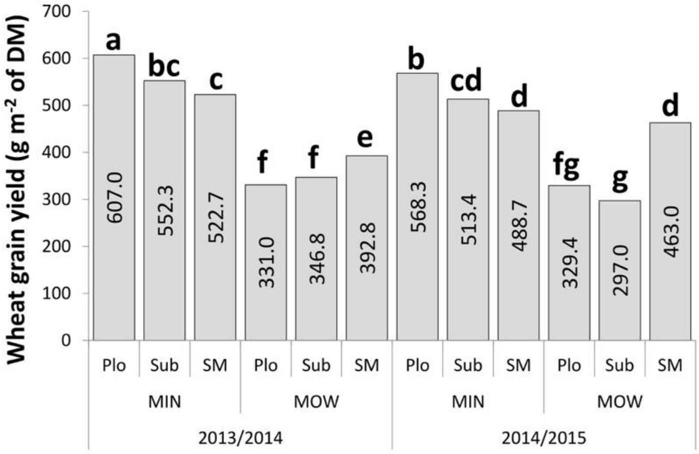


Figure 3. Canonical discriminant analysis (CDA) of the weed species based on fertilizer sources (**A**) and soil tillage (**B**) in 2013/2014 and 2014/2015 observed in durum wheat. MIN = mineral fertilization; MOW = municipal organic waste; Plo = plowing; Sub = subsoiling; SM = spad- ing machine. VIOAR = *Viola arvensis*; FUMOF = *Fumaria officinalis*; CENCY = *Centaurea cyanus*; MATCH = *Matricaria camomilla*; POLCO = *Fallopia convolvolus*; GALAP = *Galium aparine*; AN- GAR = *Anagallis arvensis*; STEME = *Stellaria media*; VERHE = *Veronica hederifolia*; PAPRH = *Papaver rhoeas*; RASRU = *Rapistrum rugosum*; CHYSE = *Chrysantemum segetum*; SINAR = *Sinapis arvensis*; SYLMA = *Sylibum marianum*; CAPBP = *Capsella bursa-pastoris*; MALSI = *Malva sylvestris*; CIRAR = *Cir- sium arvensis*; SONAR = *Sonchus arvensis*; TAROF = *Taraxacum officinalis*; CONAR = *Convolvulus arven- sis*; PHAMI = *Phalaris minor*; APESV = *Apera spica-venti*; AVEST = *Avena sterilis*; PHACA = *Phalaris canariensis*; LOLPE = *Lolium perenne*.

3.2 Durum Wheat Yield

There were significant interactions for year × fertilization source × soil tillage on the durum wheat yield (Figure 4). The durum wheat yield varied from 607.0 $g \cdot m^{-2}$ in 2013/2014-MIN-Plo to 297.0 $g \cdot m^{-2}$ in 2014/2015-MOW-Sub. Grain yield was generally higher in 2013/2014 compared with 2014/2015 (on average 457.1 vs. 443.3 $g \cdot m^{-2}$, respectively). Moreover, it was higher in MIN than the MOW fertilization source (on average 542.1 vs. 360.0 $g \cdot m^{-2}$, respectively), except in 2014/2015-SM where no differences were detected between MIN and MOW fertilization sources (Figure 4). In both growing seasons, grain yield of durum wheat was higher in the Plo tillage under the MIN fertilization source followed by Sub and SM soil tillage (on average 587.7, 532.9 and 505.7 $g \cdot m^{-2}$, respectively). Conversely, in MOW treatments, the greater grain yield was observed in SM tillage compared with Sub and Plo tillage treatments (on average 427.9 vs. 326.1 $g \cdot m^{-2}$, respectively). Durum wheat straw followed the same trend to that described for the grain yield, and it was generally higher in 2013/2014 than 2014/2015 (on average 718.8 vs. 653.9 $g \cdot m^{-2}$, respectively) and in MIN fertilizers sources compared with MOW (on average 794.9 vs. 577.8 $g \cdot m^{-2}$, respectively). Among the soil tillage practices, the straw was greater in Plo and Sub than SM in 2013/2014 (on average 911.9 vs. 747.5 $g \cdot m^{-2}$, respectively), and similar values were observed among the soil tillage systems in 2014/2015-MIN (on average 732.6 $g \cdot m^{-2}$, Table 4). Among the MOW fertilization sources, wheat straw was the highest in 2014/2015-MOW-SM compared with the other soil tillage treatments (on average 726.9 vs. 499.3 $g \cdot m^{-2}$, respectively), while the straw was similar among all soil tillage treatments (Figure 4).



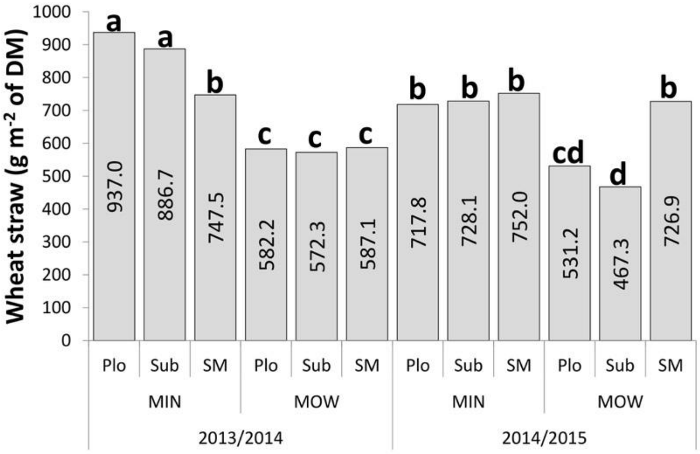


Figure 4. The interaction effect of year × fertilization source × soil tillage on the grain yield and straw of durum wheat. Values within either grain yield or straw followed by the same letter are not significantly different according to LSD (0.05). MIN = mineral fertilization; MOW = municipal organic waste; Plo = plowing; Sub = subsoiling; SM = spading machine.

4. Discussion

The results agree with those of Zanin *et al.* [38], which show a significant interaction between soil tillage regimes and fertilization sources on weed community composition in durum wheat. Similarly, Campiglia *et al.* [23] showed that the density and biomass of weeds tend to be higher under alternative tillage practices, such as subsoiler, and after organic fertilization. Conversely, low weed diversity tends to be typical of intensively managed soils, as stated by Nichols *et al.* [39], highly disturbed environments tend to be simpler than more stable ones.

The analyses showed a total of 25 different species, most of which were annual (76%) and dicotyledonous (80%), even if a significant correlation has been found between the annual monocots species and the conventionally tilled soil (plowing) [40]. Similarly, Nichols *et al.* [39] reported that annual broadleaves weeds are favored under high disturbance environments, such as conventionally tilled soil, while perennial weeds can successfully grow under low disturbance environments. It is well known that the plowing through the moldboard causes a complete overturning of the soil clods, deeply burying the majority of weed seeds, which will become dormant [41,42]. However, at the same time, another large section of weed seeds will be brought to the surface and subjected to bad weather, which will reduce their germination rate [43]. Furthermore, by plowing, the reproductive root system of some noxious perennial weed species, such as *Cirsium arvensis*, will be inactivated, reducing the presence of these kinds of perennial weeds in the community of plowed soil practices. Perennial dicots were mainly associated with alternative soil tillage, such as subsoiling and a spading machine, adopted for seedbed preparation of durum wheat. In the case of soil tillage that does not involve a complete inversion of the soil clods, such as subsoiling, the effect will tend to be less marked with regard to the germinability of the weed seeds of annual species and the survival of reproductive root systems of perennial weed species [44]. Similarly, the spading machine only worked the soil to a depth of 20 cm, thereby keeping the weed seeds in the active layer of the soil where they can easily germinate. Moreover, the presence of some perennial weed species under spading machine treatments could be explained by the effect of spading that chopped and spread the reproductive parts of the plants, such as stolons or rhizomes, resulting in an increased density.

With regards to the fertilization sources, it is well-known that different sources of fertilizer can change the efficiency and availability of nutrients in the soil and, therefore, the development of the weed community. Manipulation of crop fertilization may represent a promising agronomic means for managing weed interference in crops [13]. In general, fertilizers, especially nitrogen fertilizers, are more beneficial to weeds than crops as they show greater responsiveness to timing and application methods, as observed by Blackshaw in spring wheat [15]. In fact, nitrogen stimulates seeds of some weed species by breaking dormancy and, thus, has a considerable impact on the composition of infesting flora [44]. According to the findings of Blackshaw *et al.* [45], under high soil nitrogen availability, nitrophilous weed species were more responsive than wheat, reducing the amount of nitrogen available to the crop and increasing the competitive ability of those weeds. They may affect competition between plants, alter the density of the seed bank and affect the susceptibility to herbicides of certain weed species [46,47]. Mineral fertilizers benefit weeds with rapid growth rate, as in the case of monocotyledonous, such as *Avena sterilis* and *Phalaris* spp., and annual plants, such as *Galium aparine* and *Convolvulus arvensis*, which are well-known as nitrophilous species [20,23]. Moreover, Blackshaw *et al.* [45] observed that the application of mineral nitrogen fertilizers results in an increase of the seedbank of nitrophilous weed species resulting in a potential risk in the long-term run. On the other hand, organic fertilizers can also increase the composition of the weed community because of changing the physical and chemical properties of the soil. Although the nitrogen rate was the same between both fertilizer sources, it is conceivable that the soil nitrogen content for the crop was lower in organic source compared with the mineral fertilizer. The reduced soil nitrogen availability, based on the mineralization rate of organic materials, also favors non-nitrophilous species; therefore, a wide composition of weed community is expected [29]. This is in accordance with the findings of Hyvönen *et al.* [48], who observed a higher mean species number of weed communities in organic than conventional cropping systems. The increased weed community composition results in different weed types, for instance, dicotyledonous and monocotyledonous, that place plants in different ecological niches and cause the competition in a high biodiversity system to be lower than a single dominant weed would have. However, the fertilizer source is not the only agronomical factor affecting the community composition of weed species. The application method and timing of the fertilizer could result in changes of weed flora [49]. For example, increasing nutrient levels can reduce the abundance of weeds but select a few species that will become more competitive. Some weed species prefer N -such as *Avena fatua*- or P -such as *Solanum nigrum*-, while others are more sensitive to other micro and macro-nutrients [44]. In addition, a diffuse surface application can benefit plants that sprout in the surface part of the soil, such as most annual plants [50].

According to Siddiqui *et al.* [51], the highest productions of grain and straw were obtained in conventional management (MIN-Plo), while grain yield was always lower under organic fertilization source regardless of the soil tillage adopted. Nevertheless, the values of grain and straw in the 2014/2015 growing season in the case of MOW-SM were found to be similar to values obtained for MIN-Plo to straw but were lower for yield. These results may be related to climatic effects and, in particular, to the rainfall regime of the respective growing years. In fact, the grain yield gap observed between fertilizer sources was probably due to the high precipitation, especially during the grain filling stage, which increased the loss of mineral nitrogen via leaching, reducing the possibility to be used for the crop. This conclusion is supported by the grain yield observed in the 2014/2015 growing season when rainfall was limited and similar to the long-term period and, thus, the availability of soil nitrogen was enough to satisfy the requirements of the cereal crop regardless of the fertilizer source. Similar findings have been previously observed by Campiglia *et al.* [20]. In these conditions, spading tillage seems to ensure stable grain yield in both fertilization regimes, even if it was always lower than that observed under MIN-P. The adoption of an increased number of organic fertilizers or the integration of organic fertilizers with lower rates of mineral fertilizer could be advisable to compensate for the lack of available nitrogen.

Although the conventional management, such as plowing and mineral fertilization, has resulted in higher grain yield compared with alternative cropping systems, it seems to lead to a selective weed condition that, in the long run, could weaken the resilience of the system by gradually selecting the most competitive weed species that are more difficult to manage, as already observed in previous studies [23,52–54]. In the case of alternative management practices, such as subsoiling and organic fertilization, it is well known that they may benefit the health of the soil [23,39]. In fact, the integration of these agronomical practices contributes to synergistic outcomes that include improved soil conditions as well and more diverse weed communities. Therefore, organic fertilizer and alternative tillage, particularly spading, can be used to improve the sustainability of cropping systems in the long term [55].

5. Conclusions

The results presented in this study showed that the wheat grain yield is reduced when organic fertilizer and alternative tillage are practiced. Considering that grain yield is the main parameter that makes sense for the farmers and is the reason the wheat is cultivated, these results apparently do not make any case for moving away from traditional intensive agriculture. However, the adoption of intensive practices, such as plowing, associated with mineral nitrogen fertilization may affect weed species composition by promoting a reduction of weed community, which is mainly composed of annual competitive weeds, at the expense of the wheat yield, especially in the long-term period. Conversely, the adoption of alternative tillage practices is more favorable to increase the composition of weed species composed of a wide range of weeds that are more balanced in terms of competition with the crop and preserve the biodiversity of the agroecosystems and therefore contribute to maintaining its sustainability. In particular, the adoption of subsoiler showed the highest diversity of weed flora, even if spading tillage may represent the optimal compromise to preserve weed biodiversity and maintain satisfactory durum wheat yield. Furthermore, the mineral nitrogen, not immediately used by the wheat plants after the fertilization, remains available in the soil, stimulating the establishment of nitrophilous weeds well-known as competitive. In this sense, the adoption of organic fertilizer gradually releases nitrogen throughout the mineralization process triggered by several agro-environmental factors allowing a higher weed species composition and reducing the environmental risks due to nitrogen leaching. In particular, the combination of spading tillage with the organic fertilizer encourages weed biodiversity and is, therefore, considered to be preferable when sustainable agriculture needs to be adopted to benefit the entire agroecosystem.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10 .3390/su13137307/s1. Table S1: Analysis of variance summary of weed characteristics and durum wheat yield and straw across the field experiments in the 2013/2014 and 2014/2015 growing seasons.

Author Contributions: Conceptualization, E.R. and R.M.; methodology, E.R. and R.M.; software, R.M.; validation, E.R., R.M., V.P., A.S.L. and M.A.; formal analysis, E.R. and R.M.; investigation, R.M. and E.R.; resources, R.M.; data curation, E.R. and V.P.; writing-original draft preparation, V.P. and E.R.; writing-review and editing, V.P., E.R., A.S.L., M.A. and R.M.; visualization, E.R.; supervision, R.M. All authors have read and agreed to the published version of the manuscript.

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Chapter 2

Microbial Indices to Assess Soil Health under Different Tillage and Fertilization in Potato (*Solanum tuberosum* L.) Crop

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Sara Marinari[1], Emanuele Radicetti[2]*, Verdiana Petroselli[3], Mohamed Allam[3] and Roberto Mancinelli[3]

[1] Department for Innovation in Biological, Agro-Food and Forest Systems (DIBAF), University of Tuscia,Via San Camillo de Lellis, snc, 01100 Viterbo, Italy; marinari@unitus.it

[2] Department of Chemical, Pharmaceutical and Agricultural Sciences (DOCPAS), University of Ferrara, Via Luigi Borsari, 46, 44121 Ferrara, Italy

[3] Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, Via San Camillo de Lellis, snc, 01100 Viterbo, Italy; verdiana.petroselli@unitus.it (V.P.); mohamed.allam@studenti.unitus.it (M.A.); mancinel@unitus.it (R.M.)

***** Correspondence: emanuele.radicetti@unife.it

Abstract

Intensive agronomic practices such as deep and repeated tillage and applying high mineral fertilization rates to improve crop yields have gradually determined soil resource degradation. A study was carried out over a two-year period (2015 and 2016) to assess effects of tillage (plough; subsoil; and spading) and fertilization (mineral vs. organic) on soil health relative to carbon and nitrogen dynamics in potato crop in the Mediterranean environment. Microbial indices could be successfully used as tool for assessing soil health in terms of predictors and indicators of carbon sequestration and nitrogen availability. The microbial quotients, calculated as percentage of the microbial-C to total organic C (Cmic:Corg), was significantly higher in subsoiling than in plowing and spading soil tillage, and higher in 2016 (3.19%) than 2015 (1.72%). The activity of enzymes involved in C cycle was significantly higher in subsoiling and spading than in plowing, while acid phosphatase was positively affected by spading and arylsulfatase increased with plowing. The whole enzyme activity expressed as synthetic enzymatic index (SEI) was positively affected by subsoiling and plowing in 2015 (4254) compared to spading tillage (3934). A general decrease in soil enzyme activity in 2016 than 2015 was observed. The subsoiling in potato crop favored the immobilization of carbon and nitrogen during the wet spring–summer period. Conversely, the plowing favored the mineralization process when the spring–summer period became more dried.

Keywords: soil health; organic fertilization; soil tillage; potato crop; sustainable agriculture.

1. Introduction

Nowadays, the adoption of intensive agricultural practices, such as deep and frequent tillage associated with high mineral fertilization rate aimed to improve crop yields ensure food and human nutrition, have gradually led to the degradation of the soil resource [1]. Soil health is defined primarily from an agricultural perspective as “the soil’s fitness to support crop growth without becoming degraded or otherwise harming the environment” [2]. Based on this definition, agricultural soils are considered a complex and dynamic component and represent an essential non-renewable resource of the agroecosystems due to their benefits and functions played in the agricultural systems, therefore, their management should be a cause of intense deliberation. From an ecological point of view, more attention is currently related to soil quality indicators that consist of a range of physical, chemical, and biological characteristics based on well specified soil ecosystem functions played by soils and its components, such as nutrient cycling, soil structure and stability, and soil microbial biodiversity [3–5].

Agroecosystem management affects positively or negatively the soil characteristics, in terms of physical, chemical, biological modifications, depending on the agronomic techniques applied. Soil tillage determines variation in bulk density and porosity which affect yield and nutrient content of sweet potato. In addition, fertilization with poultry manure or NPK increases potato tuber yield, but the soil characteristics are better with organic fertilization than chemical ones [6]. The adoption of conservation soil tillage agronomic techniques can save energy, protect the soil, and contribute to reducing greenhouse gas emissions. The soil tillage operated with different machineries could affect positively the water holding, increasing the incorporation of crop residues in the soil surface with higher water content in the upper soil layer and lower evapotranspiration, lower soil temperature, and more stable soil aggregates, protection of soil erosion, nutrients and organic C accumulation near the soil surface, nitrogen leaching decreased [7]. Most of the crop cultivation came with intensive tillage operations using plow and disk harrow as primary and secondary tillage equipment with multiple fields passes to create a suitable seedbed for crop cultivation [8]. Although soil tillage had a positive influence on crop performance, intensive tillage could cause adverse effects on soil fertility [9]. Since tillage fractures the soil, it disrupts soil structure, leading to poor water-infiltration while accelerating surface runoff and soil erosion, reducing root growth [10]. Currently, reduced tillage practices aimed to reduce soil disturbance is considered one of the most effective agricultural practices to improve soil health and address environmental benefits of agroecosystems [11–13].

Organic fertilization combined with reduced soil tillage can contribute to contrast the soil degradation such as the decreasing of soil organic matter, pH alteration, cations and organic matter compounds leaching and at the same time stimulating effect on arbuscular mycorrhizal fungi [14]. In this context, the overuse of mineral fertilizers, especially nitrogen (N) could negatively affect soil quality and microbial community structure [15]. Therefore, optimal N rate and its management in the distribution are particularly important when conservation tillage practices are adopted, to avoid reduced efficiency and dangerous loss of nutrients in the environment [16–18]. The use of organic fertilizers in potato crop can determine a better use of nutrients and reduce the crop requirement of mineral fertilizers, consequently, affecting the increasing of tuber yield and their nutritional value [19].

Soil microbiological and biochemical indicators can be used to assess soil health, which has been defined as the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA-NRCS). In fact, soil microbial communities, considered an important indicator of soil health and quality, are extremely sensitive to both agricultural practices [20]. The role of the microbial fraction in mediating soil processes, and their relatively high rate of turnover, logically suggests that microbial fraction could be a sensitive indicator and early predictor of changing soil organic matter processes [21]. Soil health depends on soil biodiversity established by many soil properties and environmental conditions; therefore, its assessment in agroecosystem requires the selection of the most sensitive biological indicators to management practices [22]. The proper approach in defining soil health indicators with respect to nutrients cycling function must be holistic, but at the same time simple, so that the evaluation of soil management impact (e.g., tillage and fertilization) on soil health in a short-term experiment should consider at least the mineralization–immobilization as the two main processes regulating C storage and nutrient availability [23]. Among all soil biological properties, microbial biomass and soil enzyme activities prove to be sensitive indicators of soil health as they are measurements which rapidly respond to changes due to different management and environmental factors [24].

A better understanding of these processes could be used to improve our knowledge on soil health conditions as consequences of soil tillage and fertilizer source adopted and, thus, to support the adoption of sustainable management practices in agroecosystems. This study hypothesized that reduced tillage practices and organic fertilizer source applied to potato crop determine the development of soil microbial response toward improved soil health. The aim of this study was to assess the effects of tillage (plough; subsoil; spading) and fertilization (mineral vs. organic) on soil health with respect to carbon and nitrogen dynamic in potato crop in Mediterranean environment. In this study soil health was assessed using microbial indices as indicators of mineralization–immobilization processes as predictors of carbon sequestration and nitrogen availability in a longer period.

2. Materials and Methods

2.1 Experimental Site and Treatments

The trials were established at the Experimental Farm of the University of Tuscia (Viterbo), located approximately 80 km North of Rome (42°42′ N, 12°07′ E). The study was carried out over a two-year period (2015 and 2016 cropping seasons). The area is a typically Mediterranean climate. The climate is characterized by warm-dry summers and cool-moist winters. Historical climatic data show a mean annual rainfall of 752 mm, most of it in the period October–May, and a mean annual temperature of 14 °C, the lowest mean monthly temperature of 1.6 °C in January and the highest mean monthly temperature of 30.8 °C in August. The data of minimum and maximum temperature and the amount of rainfall were collected from the meteorological station located approximately 100 m from the experimental fields. The total precipitation was higher in 2016 than 2015 and tended to be differently distributed between the potato growing seasons. The precipitations in 2015 were mainly concentrated in the first month after potato sowing, while in 2016 were mainly distributed in the period May–June.

The monthly aridity indexes (AI) as the (precipitation)/(temperature ratio+10)) were calculated as reported by Mancinelli *et al.* [25] throughout the study periods and are reported in Figure 1.

The soil at the experimental site is of volcanic origin and classified as *Typic Xerofluvent* with the particle size distribution was 76.3% sand, 13.3% silt, and 10.4% clay, and the total organic C content 0.97%, the total organic N content 0.12%, and pH (H_2O) 6.9.

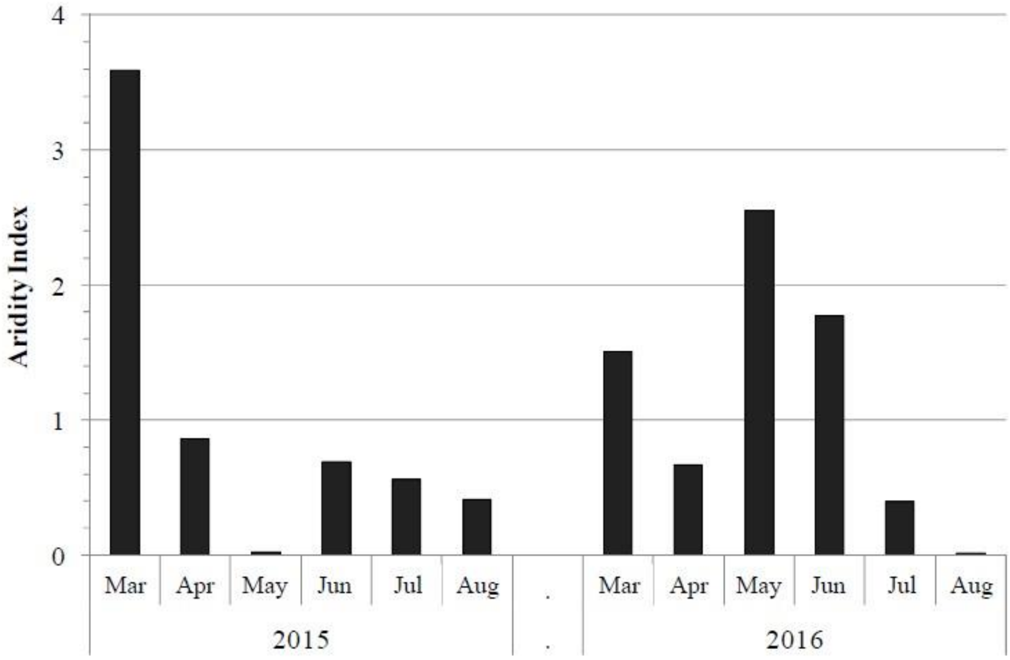

Figure 1. Aridity index throughout the periods of study in 2015 and 2016.

A cropping system based on a 2-year crop rotation [durum wheat (*Triticum durum* Desf.) - potato (*Solanum tuberosum* L.)], was established in 2013 to compare soil tillage techniques and fertilizer sources. So, the treatments consisted of three tillage systems up to 20 cm of the soil layer (mould-board plow (hereafter called P); subsoiler (hereafter called R); and spading machine (hereafter called S)) and two fertilizer sources (mineral fertilization (hereafter called M) as performed in conventional farming and organic fertilization (hereafter called O) using municipal organic waste). The applied experimental design was a randomized complete block with three replications. Both crops in rotation were simultaneously cropped in each year; therefore, the experimental field included 36 plots (2 crops x 3 soil tillage x 2 fertilizer source x 3 blocks). Each experimental plot was 60 m^2 (6x10 m) and they were separated by 3 m wide alleys to allow for equipment operations. The field setup and crop management are in detail reported by Mancinelli *et al.* [11], therefore here ahead are very briefly described only. Before seeding the potato tubers (Monalisa variety) in April the soil was tilled at 20 cm depth according to the selected treatments. The mineral fertilizer was applied twice, the first time at potato seeding (100 kg ha^{-1} of P_2O_5, 50 kg ha^{-1} of K_2O and 100 kg ha^{-1} of N) and the second time after six weeks, at hilling stage (70 kg ha^{-1} of N). The organic fertilization was totally applied at planting after soil tillage and before seedbed preparation (18 Mg ha^{-1} of mature organic waste). Irrigation water by sprinkler irrigation system was applied when soil moisture reached 65% of the soil field capacity in each experimental year and stopped two weeks before tuber harvesting.

2.2 Soil Sampling

Soil samplings were carried out during the potato crop cycle period in both years (2015 and 2016). Five soil samples per plot were randomly taken in the central area and mixed in order to obtain a uniform sample. The samples were carried out by taking the soil at a depth of 0-20 cm in each plot. The soil sample was dried at air, sieved with a 2 mm mesh sieve and stored at 4 °C temperature. Subsequently, the soil analyses were performed.

2.3 Soil Analysis and Microbial Indices

Total organic carbon (TOC) and nitrogen (TN) contents were determined using the dry combustion method with Thermo Soil NC-Flash EA1112 Elemental Analyzer (Thermo Fisher Scientific, Bath, UK). Each sample was pre-treated with a 10% HCl solution to eliminate carbonates. Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) were determined according to the fumigation–extraction method [26], using the TOC-V CSN and TNM-1 analyzer (Shimadzu, Japan). Microbial quotients were calculated as percentage of the microbial-C to total organic C (C_{mic}/C_{org}) and microbial-N to total N. The labile carbon (LC) and labile nitrogen (LN) were measured using the same equipment on non-fumigated samples and expressed as a percentage of total organic C (LC/TOC) and N (LN/TN), respectively.

The following hydrolytic enzymes, known to be a part of soil biogeochemical cycles of C, N, P and S [27], were analyzed: for carbon β-glucosidase (EC 3.2.1.21), α-glucosidase (EC 3.2.1.20), xylosidase (EC 3.2.2.27) and cellobiohydrolase (EC 3.2.1.91); for nitrogen chitinase (EC 3.2.1.30), for phosphorus acid-phosphatase (EC 3.1.3.2); for sulphur arylsulphatase (EC 3.1.6.1). Finally, the butyrate esterase (EC 3.1.1.1) was analyzed as a proxy of intracellular activity [28]. The selected enzymes activity offers a general overview of soil organic matter mineralization process subjected to the different agronomical practices applied. Enzyme activities were determined using a microplate assay [29] with fluorogenic substrates (4-MUF-β-D-cellobioside, 4-MUF-N-acetyl-β-glucosaminide, 4-MUF-β-d-glucoside, 4-MUF-α-d-glucoside, 4-MUF-phosphate, 4-MUF-sulphate, 4-MUF-7-β-d-xyloside and 4-MUF-butyrate as substrates). Fluorescence (excitation 360 nm, emission 450 nm) was measured with an automatic fluorimetric plate-reader (Fluoroskan Ascent, Thermo Fisher Scientific, Waltham, MA, USA) and readings were taken after 0, 30, 60, 120 and 180 min of incubation at 30 °C [30]. The whole enzyme activities were expressed as Synthetic Enzymatic Index (SEI) [31]. The SEI was calculated as the sum of 8 enzyme activities (β-glucosidase, α-glucosidase, xylosidase, cellobiohydrolase, chitinase, acid-phosphatase, arylsulphatase, butyrate esterase) and was expressed per unit of soil total organic carbon (SEI/TOC) or per unit of soil microbial biomass carbon (SEI/C_{mic}) [15]. The microbial quotients and the enzyme specific activities (enzyme activity per unit of C_{mic}) were used as valid indicators of the microbial efficiency in the use of energy and available nutrients [32].

2.4 Data Analysis

All data were subjected to statistical analysis by using the JMP statistical software package 4.0 (JMP Statistical Discovery, Cary, NC, USA).

The analysis of variance (ANOVA) was conducted for the two-year period applying a randomized complete block design with three blocks. A two-way factorial experimental design was performed for measured characteristics where the soil tillage and fertilization source were the treatments, and the year was considered as a repeated measure [33,34]. Means were compared adopting the Fisher's protected least significant difference (LSD) at $p < 0.05$. Correlations between C, N pools and enzyme activities of soil were performed.

3. Results

In this study no significant differences on total organic carbon and total nitrogen were registered among tillage treatments or between organic and mineral fertilization (data not shown). Conversely, the microbial quotients expressed as a percentage of microbial carbon and nitrogen to soil total organic carbon (C_{mic}/C_{org} and nitrogen (N_{mic}/N_{tot}) varied according to the cropping season x soil tillage ($p < 0.05$) and cropping season x fertilization source ($p < 0.05$, Table 1). The C_{mic}/C_{org} was significantly higher in subsoiling than in plowing and spading soil tillage regime (on average 2.66 vs. 2.35%, respectively), also showing values on average higher in the 2016 season than 2015 cropping season (on average 3.19 vs. 1.72%, respectively). In 2015 cropping season, the N_{mic}/N_{tot} was the highest in plowing followed by subsoiling and spading (2.10, 1.84 and 1.36%, respectively), while in 2016 it was high in subsoiling (3.20%), intermediate in plowing (3.96%) and low in spading (2.34%, Table 1). Regarding the fertilization source, both C_{mic}/C_{org} and N_{mic}/N_{tot} subjected to organic and mineral fertilization revealed an opposite effect in 2015 and 2016 cropping seasons, showing an increase with mineral in 2015 and with organic fertilization in 2016 (Table 1).

Table 1. Microbial quotients expressed as percentage of microbial-C to total organic carbon (Cmic/Corg) and microbial-N to total nitrogen (Nmic/Ntot). Values belonging to the same treatment with different letters in columns for tillage or fertilization effects (lower case letter), and in rows for year effect (upper case letter) are statistically different according to LSD (0.05). P= Plowing; R= Subsoiling; S= Spading; M= Mineral; O= Organic.

	C_{mic}/C_{org}				N_{mic}/N_{tot}			
	%							
	2015		2016		2015		2016	
P	1.69	bB	3.01	bA	2.10	aB	2.96	bA
R	1.81	aB	3.51	aA	1.84	bB	3.20	aA
S	1.65	bB	3.04	bA	1.36	cB	2.34	cA
M	1.74	aB	3.02	bA	1.88	aB	2.54	bA
O	1.69	bB	3.35	aA	1.65	bB	3.13	aA

The activity of enzymes involved in C cycle was significantly higher in subsoiling and spading than in plowing (Figure 2A). Conversely, acid phosphatase was positively affected by subsoiling (Figure 2B), and arylsulfatase increased with spading (Figure 2C). No effect of tillage was registered on butyrate esterase (Figure 2D). The organic fertilization source showed higher results compared to the mineral fertilization source for the activity of enzymes involved in C cycle (on average 968 vs. 881 nmol MUF g^{-1} h^{-1}, respectively, Figure 2A), acid phosphatase (on average 423 vs. 393 nmol MUF g^{-1} h^{-1}, respectively, Figure 2B), arylsulphatase (on average 67 vs. 63 MUF g^{-1} h^{-1}, respectively, Figure 2C) and butyrate esterase (on average 1798 vs. 1592 nmol MUF g^{-1} h^{-1}, respectively, Figure 2D). The whole enzyme activity expressed as synthetic enzymatic index (SEI) was affected by cropping season x soil tillage and cropping season x fertilization source ($p < 0.05$, Table 2). The SEI was positively affected by subsoiling and plowing in 2015 (on average 4254 nmol MUF g^{-1} h^{-1}) compared to the spading tillage (3934 nmol MUF g^{-1} h^{-1}). During the 2016 cropping season was observed a general decrease in soil enzyme activity than 2015 cropping season (2036 vs. 4147, respectively), even if the SEI values was greater in spading followed by subsoiling and plowing soil tillage (on average 2290, 2067 and 1752 nmol MUF g^{-1} h^{-1}, respectively; Table 2). Moreover, in both cropping seasons, organic fertilization sources showed a significant positive effect on soil enzyme activity compared to the mineral sources (on average 3254 vs. 2929 nmol MUF g^{-1} h^{-1}, respectively; Table 2).

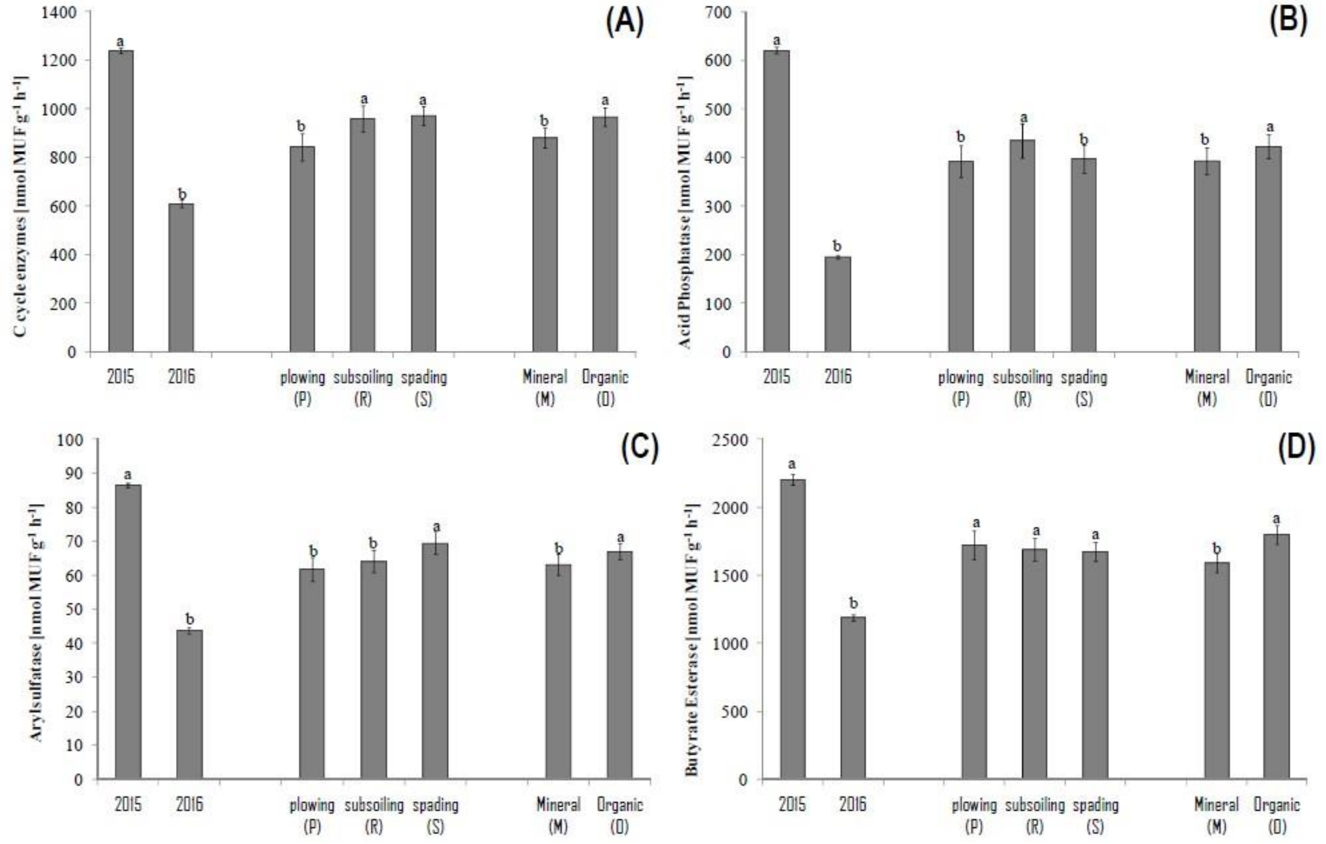

Figure 2. Soil enzyme activity in the main treatments (year of experiment, tillage and fertilization): (**A**) enzyme activities involved in C cycle, (**B**) acid phosphatase, (**C**) butyrate esterase, and arylsulfatase (**D**). Different letters indicate significant differences ($p < 0.05$), bars are standard errors ($n = 3$).

The Synthetic Enzymatic Index expressed per unit of microbial biomass (SEI/C_{mic}) was greater in 2015 than 2016 potato cropping season (on average 16.9 vs. 5.3 nmol MUF mg C_{mic}^{-1} h^{-1}, respectively) and, in 2015, it tended to be higher in spading followed by plowing and subsoiling (16.90, 19.96 and 15.85 nmol MUF mg C_{mic}^{-1} h^{-1}, respectively), while in 2016 it was high in spading (6.18), intermediate in plowing (5.21) and low in subsoiling (4.43), respectively (Table 2). No significant differences for SEI/C_{mic} were recorded between fertilization treatments (organic vs. mineral) in both years (Table 2).

Although the percentage of labile carbon form (LC/TOC) was higher in 2016 compared to 2015 potato cropping seasons (on average 0.325 vs. 0.185%, respectively), the data showed similar values among the various applied soil tillage (Table 3). Moreover, the LC/TOC was greater in organic than mineral fertilization sources in 2016 (0.338 vs. 0.313%, respectively), while no differences were detected between the fertilization sources in 2015 cropping season. Conversely, the labile nitrogen form (LN/TN) was affected by soil tillage in both potato cropping seasons showing the greater values in plowing compared to subsoiling and spading soil tillage regime (on average 0.443 vs. 0.385%, respectively).

The LN/TN ratio was generally similar in 2015 and 2016 cropping seasons, except in plowing treatment where it was higher in 2016 than 2015 (0.484 vs. 0.402%, respectively; Table 3). In addition, the labile nitrogen was significantly higher in mineral fertilization than organic one regardless of the cropping season (Table 3). Finally, no significant correlation was registered between C_{mic} and total organic carbon, as well as N_{mic} and total nitrogen. Conversely, the total amount of organic carbon and nitrogen were positively correlated with soil enzyme activities (Table 4). Moreover, microbial biomass and labile carbon were negatively correlated with single enzyme activities and SEI/C_{mic}, while labile nitrogen was positively correlated with β-glucosidase, acid phosphatase, arylsulfatase and SEI/C_{mic} (Table 4).

Table 2. Synthetic Enzymatic Index on soil mass base (SEI) and per unit of microbial carbon (SEI/C_{mic}). Values belonging to the same treatment with different letters in columns for tillage or fertilization effects (lower case letter), and in rows for year effect (upper case letter) are statistically different according to LSD (0.05). P=Plowing; R=Subsoiling; S=Spading; M=Mineral;O= Organic.

	SEI (nmol MUF g^{-1} h^{-1})			
	2015		**2016**	
P	4281.04	aA	1752.74	cB
R	4227.36	aA	2067.82	bB
S	3934.38	bA	2290.27	aB
M	4040.94	bA	1818.40	bB
O	4254.25	aA	2255.49	aB
	SEI/C_{mic} (nmol MUF mg C_{mic}^{-1} h^{-1})			
	2015		2016	
P	17.96	aA	5.21	abB
R	15.85	cA	4.43	bB
S	16.90	bA	6.18	aB
M	16.78	aA	5.32	aB
O	17.02	aA	5.23	aB

Table 3. Labile carbon expressed as percentage to total organic carbon (LC/TOC) and labile nitrogen expressed as percentage to total nitrogen (LN/TN). Values belonging to the same treatment with different letters in columns for tillage or fertilization effects (lower case letter), and in rows for year effect (upper case letter) are statistically different according to LSD (0.05). P = Plowing; R = Subsoiling; S = Spading; M = Mineral; O = Organic.

	LC/TOC				**LN/TN**			
	2015		**2016**		**2015**		**2016**	
P	0.182	aB	0.333	aA	0.402	aB	0.484	aA
R	0.187	aB	0.320	aA	0.376	bA	0.395	bA
S	0.186	aB	0.323	aA	0.384	bA	0.383	bA
M	0.187	aB	0.313	bA	0.413	aA	0.460	aA
O	0.183	aB	0.338	aA	0.361	bA	0.381	bA

Table 4. Pearson's correlation coefficient between C, N pools and enzyme activities of soil. TOC = Total organic carbon; TON= Total organic nitrogen; C_{mic}= Microbial carbon; N_{mic}= Microbial nitrogen; C_{mic}/C_{org} = Microbial quotients expressed as percentage of microbial-C to total organic carbon; SEI/Cmic= Synthetic Enzymatic Index on soil mass base per unit of microbial carbon. The significance level is *, **, ***, or ns, significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, or $p > 0.05$, respectively.

	TOC	TON	C_{mic}	N_{mic}	C_{mic}/C_{org}	Labile C	Labile N
C_{mic}	ns	ns	-				
N_{mic}	ns	ns	0.77 ***	-			
C_{mic}/C_{org}	−0.64 **	−0.50 *	0.97 ***	0.76 ***	-		
Labile C	−0.58 *	ns	0.81 ***	0.76 ***	0.87 ***	-	
Labile N	ns	ns	−0.60 *	ns	−0.56	ns	-
Cellobioidrolase	0.57 *	0.54 *	−0.77 ***	−0.54 *	−0.80 ***	−0.81 ***	ns
Chitinase	0.70 **	0.73 **	−0.76 ***	ns	−0.81 ***	−0.76 ***	ns
β-glucosidase	0.61 *	0.69 **	−0.85 ***	−0.58 *	−0.86 ***	−0.80 ***	0.50 *
α-glucosidase	ns	ns	ns	0.58 *	0.54	0.59 *	ns
Acid Phosphatase	0.64 **	0.66 **	−0.86 ***	−0.55 *	−0.89 ***	−0.85 ***	0.50 *
Arylsulfatase	0.66 **	0.72 **	−0.84 ***	−0.56 *	−0.88 ***	−0.82 ***	0.55 *
Xylosidase	0.67 **	0.70 **	−0.82 ***	−0.57 *	−0.85 ***	−0.80 ***	ns
Butyrate esterase	0.67 **	0.63 **	−0.71 **	ns	−0.78 ***	−0.79 ***	ns
SEI/C_{mic}	0.65 **	0.56 *	−0.91 ***	−0.66 **	−0.95 ***	−0.90 ***	0.50 *

4. Discussion

In this study, as expected, tillage and fertilization of potato crop did not affect the total organic carbon (TOC) and nitrogen (TON) content of soil, while the soil microbial indices and labile C and N pools resulted in very sensitive indicators of soil health variation. Even if no significant correlation between enzyme activity and total C and N content were registered, a positive correlation was found between microbial quotient or soil enzyme activity and soil labile nitrogen pool, while a negative coefficient was registered with labile carbon pool. These results confirm that microbial quotient and enzyme activity can be used as sensitive indicators of soil carbon and nitrogen dynamics after tillage and fertilization practices. In fact, it is known that microbial biomass content in a soil health assessment is useful for consistently monitoring C stock changes over time, as they are affected by agricultural management history, but relatively insensitive to recent inputs and weather changes [35]. The obtained results also showed significantly different response of soil microbial indices, such as microbial quotients and specific enzyme activity, to tillage and fertilization in the two years of experiment. It is well known that the soil microbial indices show variability over the growing season. In the different fertilization applications in terms of quantity and typology, in the methods adopted for the tilling of the soil. According to Lazcano *et al.* [36], the addition of organic fertilizer sources characterized by high moisture, available forms of N and C could affect the development of soil microorganisms confirming that soil organic mass added to the agroecosystems represent a key factor for the function and quality of soil [37–39]. Indeed, soil microorganisms react differently to the soil changes caused by the fertilizer application, especially with modification of microbial community and variation of total microbial biomass [33,34]. Similarly, Heidari and colleagues [40] showed that tillage operations play an important role in soil biological properties. Overall, sustainable approach to soil tillage is mainly oriented toward reduced tillage intensity as adopted in the minimum tillage strategy because it improves soil structure, aggregate stability, and microbial diversity [40]. Sun *et al.* [41] observed that reduced tillage combined with organic fertilization appears to be an effective agricultural strategy that enhances microbial biomass, microbial residues, and bacterial and fungal abundances. In addition, microbial residues as a fraction of soil organic carbon have higher sensitivity to farming management than to soil tillage. Additionally, Chen *et al.* [42] observed that soil tillage affected soil microbial indices at lower level than organic input. However, Choudhary *et al.* [43] highlighted that the continuous distribution of a balanced fertilization adopting organic manure combined with mineral fertilizers enhanced soil microbial indices.

An enrichment of soil enzymatic activity is usually expected in response to the following: (I) increased microbial release of extracellular enzymes and/or (II) improved soil environment by changes of physico-chemical properties [44]. In our study, the specific enzyme activities showed positive response to the plowing and suggests that the increase in hydrolytic enzymes was probably more related to higher microbial physiological capacity than to a larger biomass [32]. Conversely, in subsoiling, the increased enzymatic activity was much related to the increased amount of microbial biomass. Plowing, as with other tillage operations, produces greater aeration that incorporates previous crop residues and fertilizers, thus stimulating microbial activities and, thus, boosts the mineralization process of soil organic matter affecting soil biogeochemical processes [45]. The enzyme activities expressed per unit of microbial biomass allow to assess the metabolic *status* of the microbial community as well as variations in the stabilized extracellular enzyme activity, thus integrating the information obtained using the C_{mic}:C_{org} [46]. Indeed, soil enzymes represent the active part of soil organic components and are fully involved in all biochemical processes that happen in the soil environments and are therefore frequently used as indicators of soil quality and agroecosystem functions [20]. It is known that substrate availability is the main factor influencing both the size and activity of the soil microflora [47]. On the other hand, Carter [48] found that a wheat-fallow system reduced microbial biomass, while 4-year zero tillage increased microbial biomass compared to shallow tillage and also 3 years of direct drilling caused an increase compared to a cultivated system. He concluded that microbial biomass responded rapidly to the changes in tillage and soil management. Kabiri *et al.* [49], reported that soil microbial attributes are more sensitive to tillage practices and soil disturbance than soil organic matter content. In addition, soil microbial biomass and enzyme activity were increased independently to microbial activity, and they concluded that soil microbial attributes can be useful indicators of tillage-induced changes. Even if changes in organic carbon could not be registered in a short-term experiment, the microbial biomass was modified by subsoiling, suggesting a trend towards an immobilization of organic C in a longer period. In the 2016 potato cropping season, the increase in soil microbial quotients reflects the larger organic substrates availability for microbial growth and immobilization into microbial biomass. In addition, the decrease in specific enzyme activity suggested a reduction in the mineralization process by lower biochemical activity per unit of microbial biomass [50]. Moreover, the climatic conditions, registered in the spring–summer period of the two years, significantly affected the soil microbial biomass and activity as indicators of soil health changing carbon and nitrogen dynamics.

Since a different behavior of SEI/C_{mic} was observed between 2015 and 2016 by the different climatic trend during crop cycle, the soil biochemical activity was more sensitive than microbial quotients showing different impacts of tillage on nutrient cycling with respect to climatic conditions in spring–summer period. In particular, the obtained results suggested a positive response of microbial activity to hot and dry spring–summer period, while the amount of microbial biomass was more affected by warm and wet conditions. Moreover, among soil tillage, plowing and spading increased soil enzymatic activity per unit of microbial biomass because the physiological capacity of soil microorganisms was enhanced [32], while microbial quotients were reduced. In fact, it subsists a relation between enzymatic activities and soil tillage method applied. As reported by Bielińska and Mocek-Płóciniak [51], the enzymes tests can be good indicators depending on the employed tillage system and the enzymes stimulation activity determines changes in soil chemical conditions. Those results suggested a more intense degrading activity and lower microbial biomass growth under plowing and spading during drier season. As reported by Franzluebbers *et al.* [52], soil under conventional tillage experienced greater seasonal variation in potential mineralization, showing a sensitive response to soil temperature and moisture. Since tillage may induce changes in environmental conditions such as a decline in the proportion of water-stable soil macroaggregates, moisture and temperature [24,53], a more sensitive tillage disturbance may occur during harder season to soil microbial biomass.

5. Conclusions

In this study, the adoption of microbial indices resulted in sensitive indicators of soil health and carbon dynamic changes under different tillage and fertilization in potato cropping systems. Overall, organic fertilization modified the intensity of microbial quotient and specific enzyme activity representing a key factor for improving the soil health. Therefore, it is conceivable that the increased use of organic fertilizer also in combination with mineral fertilizer could represent an environmentally friendly practice to improve the sustainability of agroecosystems. Similarly, the results suggest that soil tillage regime modified nutrient availability. In fact, subsoiling in the potato crop favored the immobilization of carbon and nitrogen in a Mediterranean climate under wet spring–summer period, showing the need of mineral fertilization for improving nutrient availability for potato crop. Conversely, the adoption of plowing tillage, which favored the mineralization process when the spring–summer period became more dried, should be combined with organic fertilization to avoid an excessive mineralization of soil organic matter and, therefore, a pauperization of soil health.

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Chapter 3

Durum Wheat–Potato Crop Rotation, Soil Tillage, and Fertilization Source Affect Soil CO_2 Emission and C Storage in the Mediterranean Environment

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Roberto Mancinelli[1], Sara Marinari[2], Mariam Atait[1], Verdiana Petroselli[1], Gabriele Chilosi[2], Merima Jasarevic[2], Alessia Catalani[2], Zainul Abideen[3], Morad Mirzaei[4], Mohamed Allam[1]* and Emanuele Radicetti[5]

[1] Department of Agricultural and Forestry Sciences (DAFNE), University of Tuscia, 01100 Viterbo, Italy

[2] Department for Innovation in Biological, Agro-Food and Forest Systems (DIBAF), University of Tuscia, 01100 Viterbo, Italy

[3] Dr. Muhammad Ajmal Khan Institute of Sustainable Halophyte Utilization, University of Karachi, Karachi 75270, Pakistan

[4] Department of Soil Science and Engineering, Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj 31587-77871, Iran

[5] Department of Chemical, Pharmaceutical and Agricultural Sciences (DOCPAS), University of Ferrara, 44121 Ferrara, Italy

***** Correspondence: mohamed.allam@studenti.unitus.it

Abstract

At present, the role of agricultural practices on the dynamic of GHGs is being investigated worldwide. In this study, the effects of soil tillage practices (conventional vs. conservation techniques) and fertilization sources (inorganic vs. organic) on soil CO_2 emissions in durum wheat (*Triticum durum* Desf.) – potato (*Solanum tuberosum* L.) rotation in the Mediterranean area were evaluated. This study aimed to understand the changes in the soil carbon content and the soil CO_2 emissions under different soil tillage practices (moldboard plow (P), subsoiler (R), and spading machine (S)) and fertilization sources (mineral (M) and organic (O) with municipal organic waste). Soil CO_2 flux, soil water content, and soil temperature data were collected for both crops using a portable closed- chamber infrared gas dynamics system. Significant relationships were detected between soil CO_2 emissions and soil temperature and soil water content. However, these relationships were found only for durum wheat crops. Our findings indicate that including sustainable agricultural practices in wheat–potato rotation system could act as an appropriate alternative option to increase soil organic carbon, mitigate CO_2 emissions, and reduce the dependence on chemical inputs and energy.

Keywords: CO_2 emission; global warming; sustainable agriculture; wheat-potato crop rotation.

1. Introduction

Rapid population growth associated with several challenges, such as lack of food security and energy, increased emissions of greenhouse gases (GHGs), climate change, and soil degradation, have adversely impacted lives on earth [1–5]. CO_2 is a major GHG that significantly affects global warming and climate change [6,7]. Anthropogenic activities significantly affect the emissions of GHGs and global warming [8,9]. Within the framework of the European Union, the aim is to become climate neutral by 2050. This plan, therefore, foresees the need to mitigate emissions by more than double the average reduction achieved each year between 1990 and 2020 [10]. The burning of fossil fuels, deforestation, intensive tillage operations, excessive use of nitrogen fertilizer, and burning of postharvest crop residues are among the main anthropogenic activities that strongly impact the emissions of CO_2 [11,12].

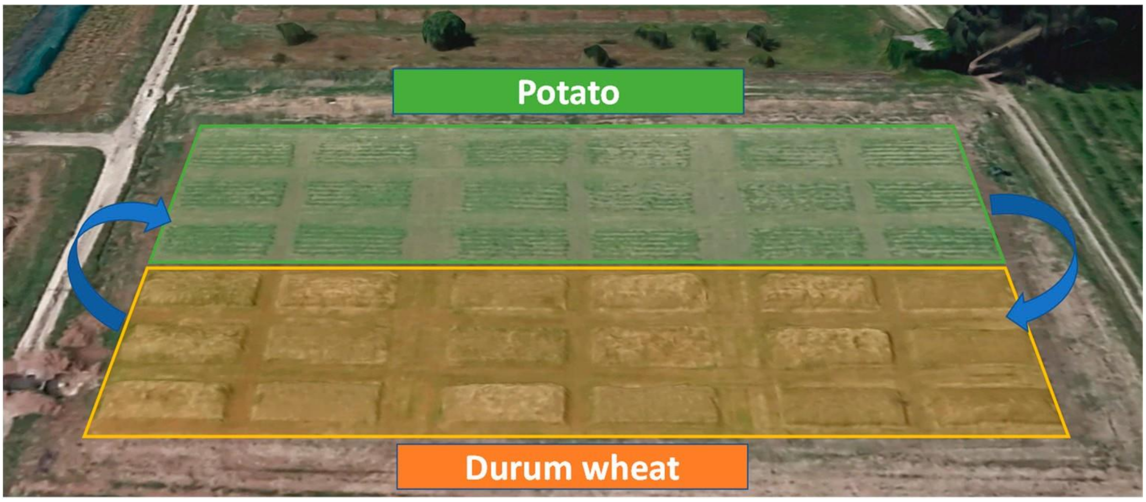
Around 10% of the total GHG emissions originate from the agriculture sector, with the largest share being attributed to emissions from cultivated land (5.27%), followed by 3.21% due to the intestinal fermentation of livestock, and others associated with the use of livestock manure (1.58%) [8,13]. The main inputs of carbon (C) into soil are through the decomposition of dead biomass, organic residues, and animal manure, while C losses mainly occur via roots and microorganisms' respiration [14]. Their relative emission flows depend on soil water content, soil temperature, nutrient availability, pH value, and land cover and management [15,16]. Agricultural activities play a crucial role in balancing the emissions of GHGs, especially CO_2 [6,17], Therefore, the development and adaptation of sustainable management practices in the agroecosystem is required to mitigate the emissions of GHGs and meet environmental concerns.

Soil tillage and fertilization practices play an important role in the dynamic of carbon and nitrogen and GHG emissions [18,19]. They represent the main agricultural practices implemented by farmers to secure high crop productivity. However, the conventional tillage system and the application of synthetic fertilizers can directly and indirectly impact environmental pollution [20,21]. Conservation tillage practices represent a fundamental pillar of sustainable farming systems [22], as plowing is the most energy-demanding process in the production of arable crops [23]. Conservation tillage techniques, such as reduced or minimum tillage (RT) and no-tillage systems, are gradually becoming reliable for farmers. In comparison with plowing, reduced tillage techniques decrease macro-aggregate destruction, and hence reduce the exposure of soil organic matter to mineralization [24]. Moreover, they improve soil health [25], enhance soil's physical properties [26], increase soil microbial biomass [27,28], positively affect soil aggregation [29], and significantly decrease greenhouse gas emissions [30,31]. Furthermore, under organic fertilization management, enhanced soil nutrient availability [32] and improved soil quality [33] have been reported. Wu *et al*. [34] suggested that using organic nutrient sources can be an effective practice to restore microbial biomass loss due to the intensive application of chemical fertilizers. Importantly, they play a positive role in climate change mitigation by soil C sequestration [32]. Using sustainable agricultural practices, such as conservative tillage techniques and the application of organic fertilizers, could be a solution to mitigate GHGs and improve crop performance. This study hypothesizes that reduced tillage practices and organic fertilization could represent a suitable and efficient strategy, in terms of carbon sequestration and reduction in greenhouse gas emissions, in a potato–durum wheat crop rotation system. In this research, we aimed to study the effects of three different tillage systems (plowing, subsoiling, and using a spading machine) and two types of fertilizer sources (inorganic vs. organic), on soil CO_2 emissions, soil organic C content, soil temperature and volumetric water content. In addition, the relationships between these soil parameters in a two-year durum wheat–potato rotation system typical of the Mediterranean area will be evaluated.

2. Materials and Methods

2.1 Study Site

The research was conducted at the "Nello Lupori" Experimental Farm of the University of Tuscia, central Italy (42°42′ N, 12°07′ E, at 310 m a.s.l.) in two growing seasons, 2021 and 2022 (Figure 1). The site is characterized by a typical Mediterranean climate with warm humid winters and hot dry summers. The average temperature is 14.5 °C, with minimum temperatures just below 0 °C in February and maximum temperatures above 35 °C in July. The average annual rainfall is 752 mm (last 30 years), mainly concentrated from September to May (569 mm). The soil of the experimental area is of volcanic origin and classified as *Typic Xerofluvent* [35]. The particle size distribution of the soil in the surface horizon (depth 0–30 cm) is defined by 76.3% sand, 13.3% silt, and 10.4% clay; therefore, it is sandy soil-clay. The soil had 0.15% total nitrogen (Kjeldhal method), 0.97% total organic content of C (Lotti method), a pH of 6.9 (water, 1:2.5), EC 2.5 dS m^{-1}, 33 mg kg^{-1} available phosphorous (Olsen extraction method) and 575 mg



kg−1 exchangeable potassium (K+, extracted using 1.0 N neutral).

Figure 1. Experimental field plan of durum wheat–potato rotation.

2.2 Field Setup

The experiment was conducted on a biennial rotation, typical of the study area, between durum wheat and potato, to compare different tillage practices and fertilization events. The treatments consisted of: (I) three soil tillage systems (conventional with moldboard plow (P), reduced tillage with a subsoiler (R), and reduced tillage with a spading machine (S)) and (II) two fertilization sources (mineral fertilizer (M) and organic (O) with municipal organic waste). The two main crops in rotation were simultaneously cropped every year. For each crop, the treatments were replicated three times according to a randomized complete block design. The experimental field included 36 plots (2 crops x 2 fertilization sources x 2 tillage managements x 3 blocks). Each plot had a size of 60 m^2 (6x10) m and 5-m-wide alleys separated all plots of the experimental field in carrying out all farming operations.

The tillage practices (P, R and S) were employed at 30 cm depth. The durum wheat, variety *Antalis*, was sown in late November to early December in both growing seasons by means of a plot seeder at a sowing density of 400 grains per m^2. A potato crop, *Levante* variety, was sown manually with a density of 7.4 plants per m^2 on 8 April 2021 and 11 April 2022, respectively. The nitrogen requirement of wheat, in the mineral application, was divided into two applications, 50% in the tillering and 50% in stem elongation, for a total of 100 kg ha^{-1} of N. The demand, in organic management, was met by 25 t ha^{-1} of compost from municipal solid waste (ACEA Ambiente, Orvieto Italia). The mineral fertilization of the potato has been divided into two applications, one for sowing with 100 kg ha^{-1} of P_2O_5, 50 kg ha^{-1} of K_2O and 100 kg ha^{-1} of urea, and a second during the tamping with 70 kg ha^{-1} of ammonium nitrate. For organic fertilization, however, only one application was carried out before sowing with 18 t ha^{-1} of compost from municipal solid waste (ACEA Ambiente, Orvieto Italia). The field setup and crop management are reported in detail by Mancinelli *et al.* [36]. The potato field was irrigated based on local weather conditions and irrigation was stopped two weeks prior to harvesting. The wheat was threshed on 14 July 2021, and 21 June 2022, and the potato was harvested on 9 August and 26 July for the first and second crop cycles, respectively.

2.3 Data Collection

Meteorological data for the 2021 and 2022 growing seasons were collected from a meteorological station 500 m from the experimental site. Soil temperature, soil water content, and soil CO_2 flux data were collected for entire crop cycles using a portable closed-chamber infrared gas dynamics analysis system following the methodology of Mancinelli *et al.* [37]. Measurements were collected every three weeks (±2 days) in each durum wheat growing season, while measurements were collected every two weeks (±2 days) in each potato growing season. The measurements were always performed between 9 and 10 AM, and after 2 days in the case of rainfall events. A non-stationary flow-through chamber (SRC-1, PP Systems, Stotfold, UK) with a volume of 1334 cm^3 and a coverage area of 78.5 cm^2 was used to perform soil CO_2 emission measurements [37]. The soil CO_2 readings were taken by placing the instrument's reading bell in a permanent PVC cylinder positioned in a central area of each plot and kept free from any weeds. The amount of soil CO_2 emissions was estimated by fitting a quadratic equation to the ratio of the increase in CO_2 concentration to the elapsed time. The values obtained were considered the net carbon mineralization, since the autotrophic component was included. The estimate was determined by considering two consecutive measurements in linear interpolation and their integration over time (trapezium rule) as previously adopted by Mancinelli *et al.* [38]. The soil temperature was measured with an STP1 probe connected to the EGM-4 instrument at a depth of 5 cm and at the same time the volumetric water content of the soil was measured at a depth of 0–30 cm with a TDR 300 instrument (Spectrum Technologies, Inc. Plainfield, IL, USA).

For the analysis of total organic carbon (TOC) and total nitrogen (TN) in the soil, five samples were taken for each plot (0–20 cm soil depth), then the samples were air dried and passed through a 2-mm sieve and stored at a temperature of 4 °C. The TOC and TN of the soil samples were determined using an elemental analyzer (Thermo Soil NC-Flash EX1112). The C content in the epigeal samples of wheat and potato crops was also determined using the same elemental analyzer. The plant samples were taken in each plot, in the ripening state, on a double row of 1 m^2 for the wheat, and on a single row of 2 m^2 for the potato. Subsequently, they were dried in an oven at 70 °C until completely dry and powdered. Lastly, the data obtained (C input) were related to the relative CO_2 emissions from the soil (C output) to determine the C input/C output ratio [39].

2.4 *Statistical Analysis*

All the data were analyzed using JMP 4.0 software. Two-way factorial analysis of variance (ANOVA) was applied to evaluate the effects of soil tillage practices and fertilizer source ad their interactions on CO_2 emissions and other soil parameters, where years were considered as a repeated measurements across time [40]. Fisher's test (LSD) was performed to examine the differences between mean values. Differences at $p < 0.05$ were considered statistically significant. Finally, a second-order polynomial regression was used to relate data on soil CO_2 emissions under different treatments (soil tillage and fertilization managements) with soil temperature and soil water content (Tables S1 and S2).

3. Results

3.1 *Weather Conditions during Durum Wheat–Potato Crop Rotation*

The 2020/2021 and 2021/2022 growing seasons differed greatly in terms of rainfall and temperature (Figure 2). The years 2020/2021 were wetter compared with 2021/2022 (770 vs. 177 m, respectively) except the period from mid-March to the beginning of April. Conversely, 2022 was dry or semi-arid during the whole season, except in April, when wet conditions were recorded.

Minimum and maximum temperatures tended to gradually increase from March to June (Figure 2). In 2021, the maximum temperature was about 14 °C degrees at the beginning of measurements in mid-March and reached 26 °C degrees in the beginning of June, when the last measurement was recorded. However, the maximum temperature was about 15 °C degrees at the beginning of measurements in mid-March and reached 31 °C degrees in the beginning of June 2022, when the last measurement was recorded.

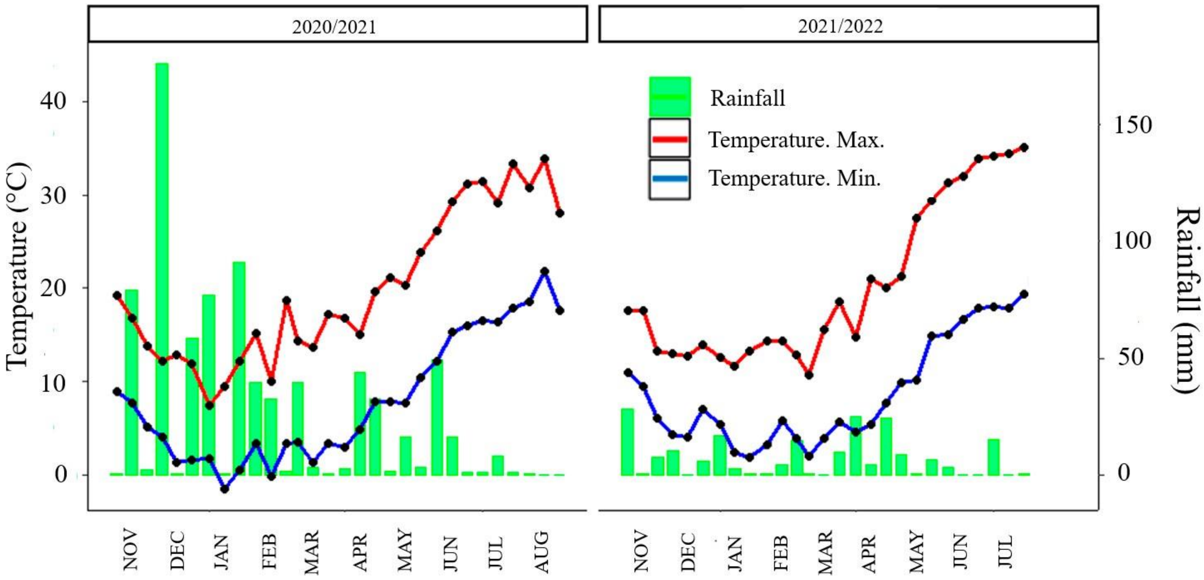


Figure 2. Minimum and maximum temperatures (°C), and rainfall (mm) at the experimental site, throughout the periods of study in 2021 and 2022.

3.2 Soil CO_2 Emissions, Temperature and Volumetric Water Content during the Crop Rotation

3.2.1 Effect of Treatments on Durum Wheat

The results showed a similar trend for CO_2 emissions in both growing seasons, where it gradually increased from mid-March and reached the highest value by the end of April, and then gradually decreased until the beginning of June. In both growing seasons, soil CO_2 emissions differed between soil tillage treatments at the end of April, when the highest values were recorded. In 2021, soil CO_2 emissions observed in P and R soil tillage treatments were similar at end of April, even if the values observed were lower than emissions measured under S treatment. At the same time in 2022, R showed significantly lower emissions in comparison to those in S and P. In addition, compared to P, lower emissions were recorded in R at the beginning of June in both growing seasons.

Soil water content during the durum wheat growing seasons showed different trends under different tillage practices in both growing seasons (Figure 3).

Compared to conventional tillage, higher soil water content was observed under R treatment at all timepoints in 2020/2021. In 2021/2022 growing seasons, soil water content showed a similar trend to that observed in 2020/2021, even if the main changes were observed at the beginning of April, when the highest values were observed: R followed by S and P (Figure 3). From mid-March till the end of April, soil water content tended to be higher under both conservation tillage practices in comparison with P, even continuing to mid-May in 2021. No significant differences were detected between soil tillage systems at the beginning of June in both growing seasons.

Soil temperature tended to increase from the beginning of April until the end of the measurements in both growing seasons, even if the soil temperature growth was faster during the 2021/2022 growing season. Significant differences in soil temperature were observed from the end of April until the start of June in 2021, where higher soil temperatures were observed under both R and S tillage practices compared to P (Figure 3). No differences were observed among the soil tillage practices in the 2021/2022 growing season, except at the end of April, where P showed the highest values of soil temperature.

Mineral fertilizers tended to have lower values compared with organic fertilizer in both growing seasons, (on average 0.45 and 0.47 vs. 0.47 and 0.37 g m^{-2} h^{-1} in 2021 and 2022, under M and O fertilization sources, respectively). The greater differences between M and O treatments were more obvious in 2022, even if O produced significantly higher CO_2 emissions only at beginning of April compared to M. No significant differences were detected for soil temperature and soil water content between fertilizer sources during the study period in 2022.

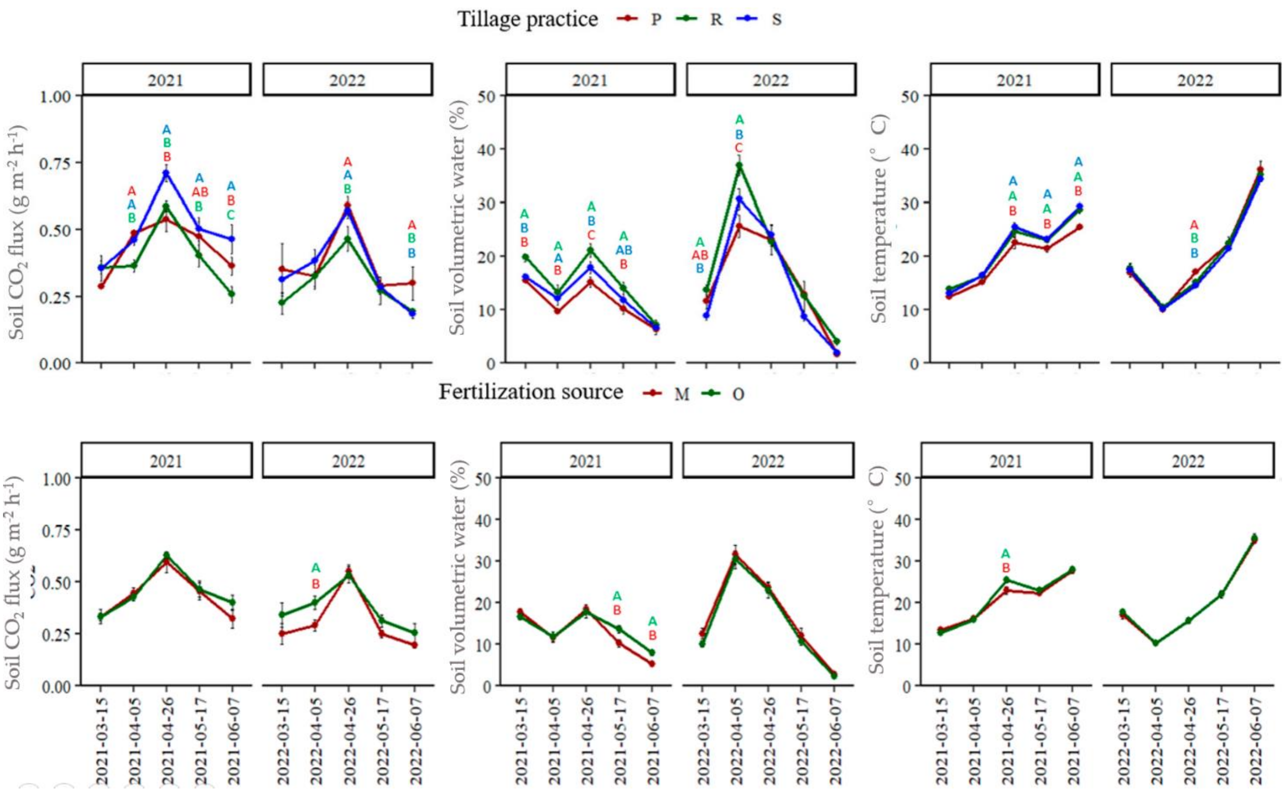


Figure 3. Soil CO_2 emissions, soil temperature, and soil volumetric water content under the three soil tillage practices: plowing (P), subsoiling (R) and spading (S), and under inorganic (M), organic (O) fertilization sources during 2021 and 2022 durum wheat growing seasons. Bars represent standard error. No letters mean that differences between the means were not significant ($p > 0.05$).

3.2.2 Effect of Treatments on Potato Crop

According to the results, soil CO_2 emissions showed various trends under the different tillage practices in both potato growing seasons. In 2021, subsoiling showed a higher peak in June than other tillage systems (on average 0.71 vs. 0.58 and 0.54 g m^{-2} h^{-1}, respectively). Conversely, in 2022, soil CO_2 emissions were always lower in R compared with P and S tillage practices (on average 0.44 vs. 0.54 and 0.59 g m^{-2} h^{-1}, respectively).

The volumetric water content of the potato was different in the two seasons, especially in the periods close to tuber sowing and harvesting. In 2021, subsoiling tended to show high values than other tillage practices, even if the greater differences were detected in July (15.66 vs. 12.25 % and 10.44 % in R, P, and S, respectively). In 2022, soil volumetric water was the highest in subsoiling compared with plowing and spading, even if the differences were observed only at the beginning of July, while in the remaining period no differences were detected among soil tillage treatments (Figure 4). In both potato growing seasons, soil temperature showed similar trends among the soil tillage treatments; it ranged from 20 °C to 30 °C during the monitoring period.

Soil CO_2 emission had similar trends between O and M fertilization sources, even if in both potato growing seasons, the O treatments tended to show high values compared with the M treatment (Figure 4). The same period, soil water content showed a similar trend during the two growing seasons, except in 2021, when organic fertilization showed a higher value compared with mineral fertilization in the measurement period (on average 13.08 % vs. 11.24 %, respectively).

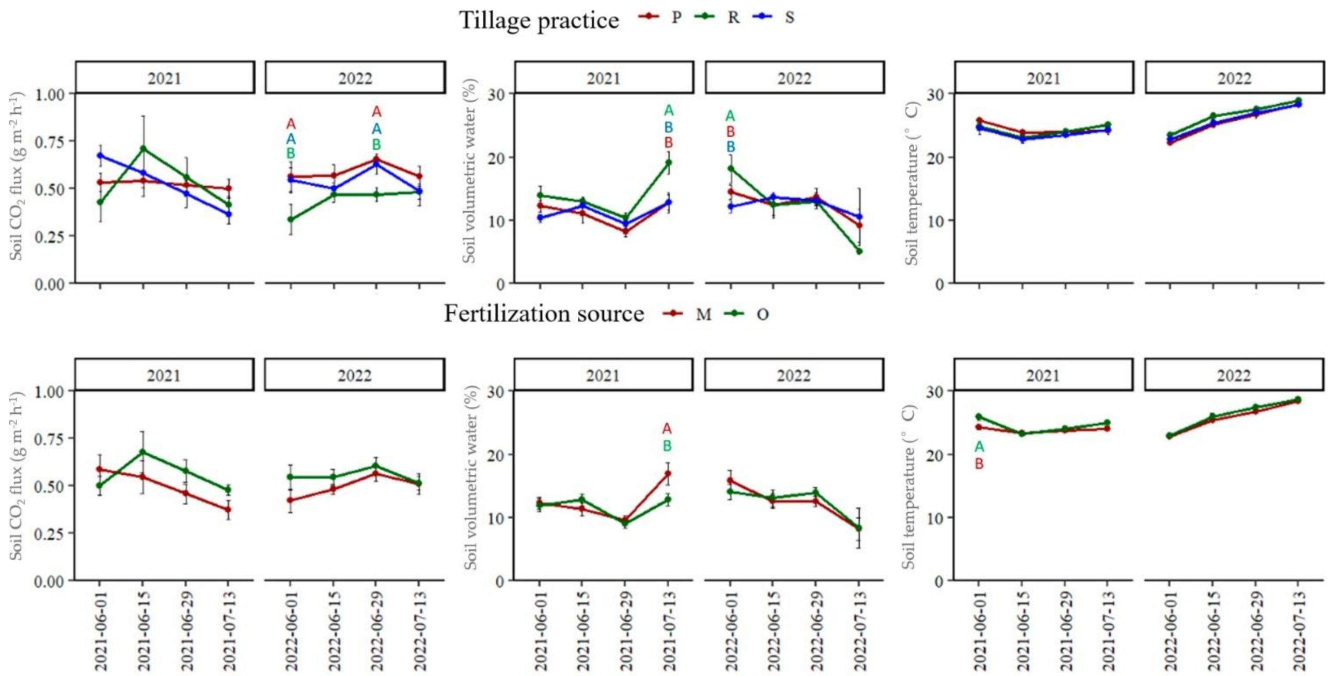


Figure 4. Soil CO_2 emissions, soil temperature, and soil volumetric water content under the three soil tillage practices: plowing (P), subsoil (R), and spading (S), and under inorganic (M), organic (O) fertilization sources during the 2021 and 2022 potato growing seasons. Bars represent standard error. No letters mean that differences between the means were not significant (p > 0.05).

3.2.3 Regression Analysis between Soil CO_2 Emissions with Temperature and Soil Water Content

The regression analysis between soil CO_2 emissions against soil temperature and soil volumetric water content were carried out for both crops, but since no differences were detected in the potato crop, only the results for durum wheat are reported (Figure 5). The results showed that the CO_2 emission rate was significantly dependent on volumetric water content under subsoiling and spading tillage practices only (Figure 5A). The highest level of CO_2 emission was 0.55 g m^{-2} h^{-1} (R^2= 0.3296; p< 0.001) and was detected at 21% volumetric water content for the spading tillage system compared to subsoiling (0.48 g m^{-2} h^{-1}; R^2= 0.2883; p< 0.001) at 28% volumetric water content.

Regarding the fertilization source, the results also showed the highest level of CO_2 emissions at the optimal soil moisture level in organic fertilization (0.49 g m^{-2} h^{-1}; R^2= 0.1671; p< 0.0001) compared to the mineral source (0.45 g m^{-2} h^{-1}; R^2= 0.1305; p< 0.0001), with both at 23% volumetric water content (Figure 5B). In addition, soil temperature and water content showed significant relationships with soil CO_2 emissions under both fertilization sources during wheat growing seasons (Figure 5B, D). However, under equal conditions of soil water content and soil temperature, higher soil CO_2 emissions were obtained in organic fertilizer compared to those in inorganic fertilizer. Soil temperature and water content explained 9–16% of the variability in CO_2 emissions under different fertilization sources during wheat growing seasons.

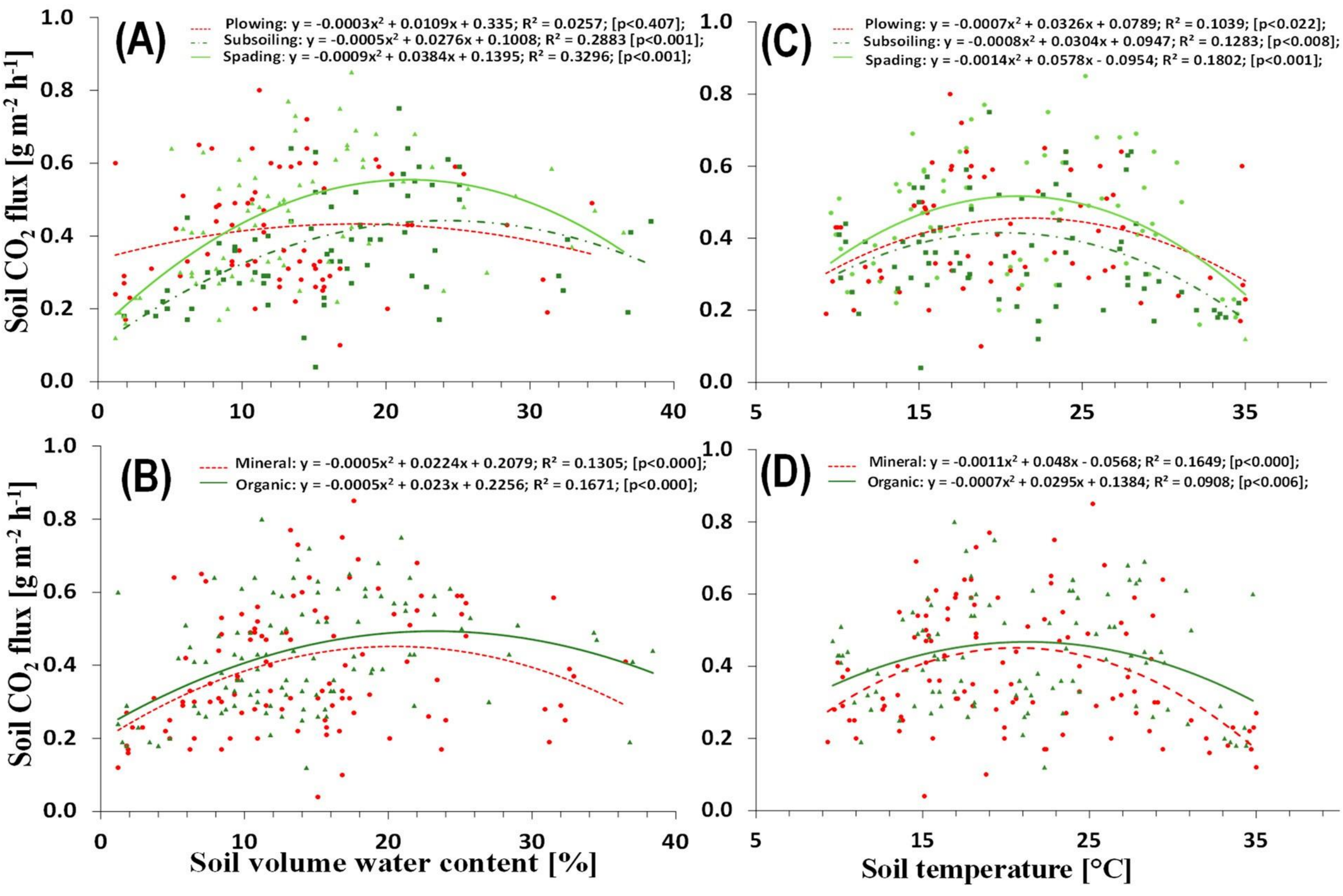


Figure 5. Soil CO_2 flux plotted against soil volumetric water content and soil temperature under soil tillage practices: plowing (P), subsoiling (R), and spading (S), and under inorganic (M), organic (O) fertilization sources for both the 2021 and 2022 durum wheat growing seasons. Soil CO_2 flux plotted against soil volumetric water content under different tillage (**A**) and different fertilizers (**B**). Soil CO_2 flux plotted against soil temperature under different tillage (**C**) and different fertilizers (**D**).

The analysis of polynomial regression also showed the dependence of the CO_2 emission rate on soil temperature. The highest rate of CO_2 emission was related to spading (0.50 g m^{-2} h^{-1} at 21 °C soil temperature; R^2= 0.1802; *p*< 0.001), the intermediate value under plowing (0.46 g m^{-2} h^{-1} at 23 °C, R^2= 0.1039; *p*< 0.022), and the lowest value under subsoiling (0.38 g m^{-2} h^{-1} at 19 °C; R^2= 0.1283; *p*< 0.008) (Figure 5C). Moreover, significant relationships were noticed under both fertilization sources. There was a higher value under organic fertilizer (0.47 g m^{-2} h^{-1}; R^2=0.908; *p*< 0.006) compared to the mineral fertilizer (0.45 g m^{-2} h^{-1}; R^2= 0.1649; *p*< 0.0001, Figure 5D).

3.2.4 Regression Analysis between Soil CO_2 Emissions with Total Organic C and Total N

A linear regression was conducted for both crops, but significance was found only in the wheat crop data (Figure 6). The results of the linear regression show that there is a close relationship between the soil CO_2 flux emissions and the total organic carbon and the total nitrogen in the soil for wheat only. Significant relationships between soil CO_2 emissions and the soil nitrogen content under different soil tillage and fertilization managements were observed. However, significant relationships between soil CO_2 emissions and the soil carbon content (C%) were found only under fertilization treatments. Interestingly, a significant negative relationship between CO_2 emissions and C% was only detected under organic management (R^2=0.2376; *p*< 0.039).

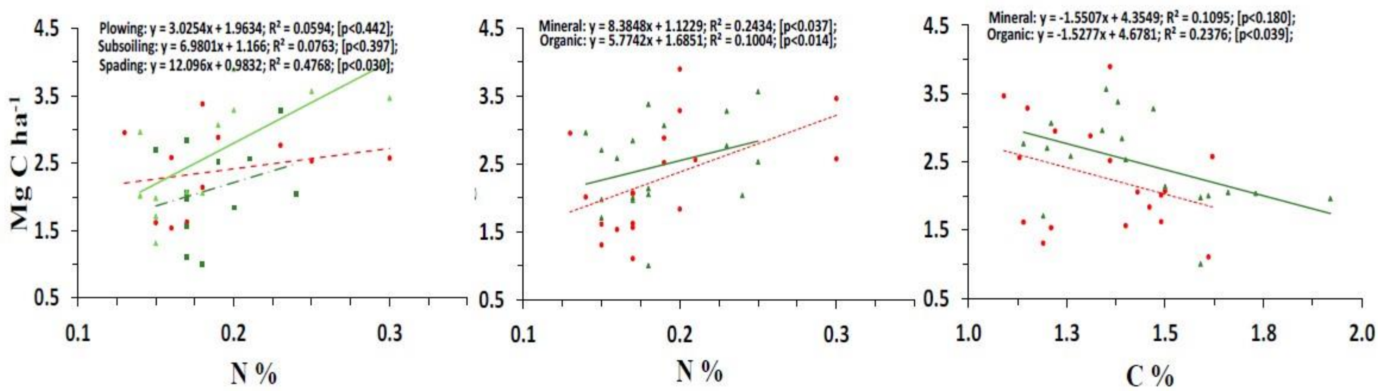


Figure 6. Soil CO_2 flux plotted in the two fertilization (Mineral - - - and Organic —) and three soil tillage (Plowing - - -, Subsoiling —, and Spading - - -) against total carbon (C%) and total nitrogen (N%) during wheat crop seasons.

However, significant positive relationships were detected between soil CO_2 emissions and the soil nitrogen content (N%) under both fertilization sources. This relationship was stronger under M (R^2= 0.2434; *p*< 0.037) than O (R^2= 0.1004; *p*< 0.014). The strongest association between soil CO_2 emissions and the soil nitrogen content (N%) was detected under the spading tillage system (R^2= 0.4768; p< 0.030).

3.2.5 Agroecosystem Carbon Balance

The C inputs were influenced by the growing seasons and fertilization source. Higher C inputs were obtained in 2021 than 2022 for both crops. Under organic management, durum wheat showed higher C inputs by biomass compared with mineral fertilizer (Table 1), and the same trend was observed in the potato crop (Table 2). Regarding the influence of soil tillage on C inputs, significant effects were only observed in potato cultivation, which has shown higher values in plowing (0.907 Mg C ha^{-1}) and spading (0.910 Mg C ha^{-1}) than in subsoiling (0.844 Mg C ha^{-1}, Table 2).

Table 1. The carbon balance in the wheat crop and the effects related to soil tillage practice, fertilization source and year. Different letters for each main effect indicate statistically significant differences according to the LSD test (p< 0.05). No letters mean that differences between the means were not significant (p > 0.05).

	C Input by Biomass [Mg C ha^{-1}]		C Output by CO_2 Emission [Mg C ha^{-1}]		C Input/C Output [Mg C ha^{-1}]		CO_2 Emission/Grain [Mg C ha^{-1}]	
Soil Tillage								
Plowing	5.139		2.385	b	2.242	b	0.973	a
Subsoiling	5.660		2.126	c	3.010	a	0.769	b
Spading	5.583		2.617	a	2.331	b	1.043	a
Soil Fertilization								
Mineral	5.236	b	2.274	b	2.545		0.783	b
Organic	5.685	a	2.478	a	2.510		1.074	a
Year								
2021	5.612	a	2.966	a	1.929	b	1.381	a
2022	5.308	b	1.786	b	3.127	a	0.476	b

Table 2. The carbon balance in the potato crop and the effects related to soil tillage practice, fertilization source, and year. Different letters for each main effect indicate statistically significant differences according to LSD test ($p < 0.05$). No letters mean that differences between the means were not significant ($p > 0.05$).

	C Input by Biomass [Mg C ha^{-1}]		C Output by CO_2 Emission [Mg C ha^{-1}]	C Input/C Output [Mg C ha^{-1}]		CO_2 Emission/ Dry Tuber [Mg C ha^{-1}]		CO_2 Emission/ Fresh Tuber [Mg C ha^{-1}]	
Soil Tillage									
Plowing	0.907	a	2.301	0.406		4.997		0.773	
Subsoiling	0.844	b	2.156	0.426		5.535		0.880	
Spading	0.910	a	2.220	0.419		5.643		0.887	
Soil Fertilization									
Mineral	0.329	b	2.073	0.171	b	4.597		0.714	
Organic	1.445	a	2.378	0.663	a	6.187		0.979	
Year									
2021	0.967	a	2.352	0.424		3.000	b	0.550	
2022	0.808	b	2.099	0.410		7.783	a	1.143	b

C outputs were affected by all factors for the durum wheat crop. C outputs were higher in 2021 than 2022 (2.966 vs. 1.786 Mg C ha^{-1}, respectively), and when using organic fertilizer compared to mineral fertilizer (2.478 vs. 2.274 Mg C ha^{-1}, respectively). In addition, the highest C outputs in durum wheat were observed using spading tillage followed by plowing, while C outputs were the lowest under subsoiling (Table 1). No significant differences were found on C outputs for the potato crop.

The C input/C output ratio was influenced differently between both crops. For the potato crop, the ratio was only affected by fertilization source, which was higher with an organic source (0.663 Mg C ha^{-1}) than mineral (0.171 Mg C ha^{-1}). On the other hand, for wheat cultivation, the ratio varied between growing seasons; it was higher in 2022 (3.127 Mg C ha^{-1}) than in 2021 (1.929 Mg C ha^{-1}). In addition, the ratio was affected by the soil tillage system, where subsoiling showed a significantly higher value (3.010 Mg C ha^{-1}) compared to other tillage practices (2.242 Mg C ha^{-1} for plowing and 2.331 Mg C ha^{-1} for spading, Table 1).

The ratio of CO_2 emissions from wheat grain yield (Table 1) had significant effects from all treatments and different growth years. The C output/grain was higher in 2021 than in 2022 (respectively, 1.381 Mg C ha^{-1} and 0.476 Mg C ha^{-1}) and higher in organic fertilization than mineral fertilization: 1.074 Mg C ha^{-1} towards 0.783 Mg C ha^{-1}, respectively. In addition, the subsoiling practice was distinguished from other tillage practices with a value of 0.769 Mg C ha^{-1}, significantly less than plowing (0.973 Mg C ha^{-1}) and the of the spading machine (1.043 Mg C ha^{-1}). However, the only factor significantly that influenced the CO_2 ratio emission/production in potato crop was the growing season, where 2021 showed lower values (3.000 Mg C ha^{-1} and 0.550 Mg C ha^{-1} for fresh and dried tubers, respectively) than 2022 (7.783 Mg C ha^{-1} and 1.143 Mg C ha^{-1} for fresh and dried tubers).

4. Discussion

The results of this work showed that agricultural practices play a significant role in dynamic of soil carbon and CO_2 emissions, which is consistent with previous research such as Radicetti *et al.* [41] and Smith *et al*. [42]. The effects of various tillage practices were stronger than those of fertilization sources on soil CO_2 emissions during wheat growing seasons. Although soil CO_2 emissions represent the result of very complex interactions between all biotic and abiotic factors of the agroecosystem, this finding is very important for utilizing natural resources in a sustainable way while having satisfactory agricultural production, minimal soil CO_2 emissions into the atmosphere, and maximal soil carbon sequestration by which climate change could be mitigated. Conservation tillage practices are well known to reduce the soil disturbance compared to that caused by plowing, promoting the right balance between micropores and macropores and slowing the rate of carbon oxidation. Therefore, any effort to decrease tillage intensity and maximize residue return should result in carbon sequestration for enhanced environmental quality [43]. However, regardless of the treatments, CO_2 emissions during durum wheat cultivation followed the same trend in both years, despite extremely different climate conditions. During the wheat growing season, soil CO_2 emissions tended to increase from mid-March till the end of April as air temperature rose, and then CO_2 emissions decreased until the beginning of June. A reduction in air temperature was observed in mid-April 2021 and the beginning of April 2022, followed by a raise in air temperature until the end of measurements during wheat seasons.

No significant differences were noticed in soil CO_2 emissions for the treatments (tillage or fertilization) in potato cropping season. Moreover, CO_2 emissions from organic sources tended to be higher in both growing seasons. According with the findings of Li and colleagues [44], soil CO_2 emissions may also result from substrate-induced priming, stimulating the decomposition of native soil C. It is well known that the availability of nutrients is essential for the vital processes of the soil. Therefore, the natural content of N and C in soil and fertilizer inputs plays a key role [18]. Organic fertilizers and compost increase SOC pools more than mineral fertilizers, so these could have a higher impact on microbial activity [45,46]. Soil tillage practices showed seasonal differences in CO_2 fluxes in potato crop cycles for 2021 and 2022. In 2021, it was observed that there was a tendency under both conservative tillage systems to reduce the emissions of CO_2 in comparison with conventional tillage [47,48]. Stable emissions were observed throughout the potato growing seasons under plowing. However, in 2022 emissions from subsoiling were significantly lower than the conventional practice. In fact, reduced tillage practices limited the number of macro-pores, which limit the presence of air and therefore decrease CO_2 emissions [48]. Nevertheless, the results of this work suggest that the subsoiling tillage practice (R) could lower soil CO_2 emissions under both crop species. This is consistent with other work stating that minimum tillage practices reduced CO_2 emissions and facilitated soil carbon sequestration [49,50]. Moreover, it was reported that conservative management can be a means of conserving soil moisture and can improve water-use efficiency [51,52]. In both growing seasons of wheat, the soil water content tended to be higher under conservation tillage practices compared to the conventional system.

Significant relationships were detected between soil temperate and soil water content and soil CO_2 in the study. These relationships were found only for durum wheat. A significant association between soil CO_2 and soil water content was detected only under both conservation tillage practices during wheat growing seasons. However, the association of soil CO_2 with soil temperature was detected under both conventional and conservation tillage practices. Moreover, a stronger relationship was observed between CO_2 and soil water content than with soil temperature under different soil tillage practices. In this study, soil temperature explained 10%-18% of the variability in CO_2 emissions under different soil tillage practices during wheat growing seasons. Soil water content explained 29%-33% of CO_2 emission variability under conservation tillage practices. Despite having the same soil water content and soil temperature, higher soil CO_2 emissions were observed in the spading tillage system compared to subsoiling and plowing. It is often reported that soil temperature is a key regulator of soil CO_2 emissions [53]. However, soil temperature had no significant relationship with soil CO_2 emissions during potato growing seasons. This might be related to the high soil temperature (more than 20 °C) from the start of measurements in June till mid-July.

In addition, no significant difference was detected between treatments (soil tillage or fertilization managements) on soil temperature during potato growing seasons, except at the beginning of June in 2021 only between fertilization sources. Although previous studies suggest as conservation tillage practices associated with soil cover contribute to reduce high soil temperatures [31,41], in this study the previous crop residues (wheat straw) are incorporated into the soil, causing a lack of insulation by residue that reduced the effects on soil temperature, determining similar values of the conventional tillage in according with the findings of Shen et al. [54]. In addition, potato hilling performed twice in all plots before the canopy closure homogenized the variation in soil temperature among treatments as observed by Nyawade *et al*. [55]. At the beginning of June 2021, the highest precipitation value was recorded during the time of measurements (more than 10 mm). High rainfall might be related to high soil respiration in that time of first measurements [56]. Moreover, soil water content had no significant relationship with soil CO_2 emissions during potato seasons. It might be related to the fact that the potato crop was under an irrigation system, which is the traditional practice in the study area.

The highest C input/C output ratio and the lowest value of the CO_2 emission/grain ratio were related to subsoiling tillage system compared to the other two systems in the wheat cropping system, but the input values from biomass were not significantly different from other soil tillage treatments. These data suggest that the subsoiling tillage not only produces the same crop yield as conventional practices, but it can also increase the carbon content in the soil and requires lower CO_2 emissions for the same production. This agrees with other studies, which state that conventionally tilled soils have higher emissions and lower amounts of carbon in soil [47,48].

The results for wheat concerning fertilization showed no significant differences between the C input/C output ratios in the two fertilizer sources, but the CO_2 emission/grain ratio was higher in organic fertilization. This result can be explained by the weak relationship found between CO_2 emissions and nitrogen in the soil, caused by a possible unbalance in the C/N ratio.

The results for potato showed significance mainly in CO_2 emissions due to biomass, where plowing and spading yielded statistically higher or similar results than subsoiling. Similar results were obtained by Mancinelli *et al*. [39]. The subsoiling could disadvantage the growth of the potato due to the greater apparent density of the soil compared to other tillage practice [57]. In the field of fertilization, the carbon input was lower in organic fertilization; In the C input/C output ratio, organic fertilization obtained higher results than mineral fertilization, but still below the value 1.

To conclude, greatest significance in potato CO_2 emission occurred between years. This result could be explained by the fact that the crop was grown under irrigation, which could have flattened any differences between treatments. SOC accumulation depends on several constitutional factors of the soil, including soil humidity and temperature [58]. Humidity is the most important soil parameter for gas emissions, as it directly controls the processes of microbial activity [15].

5. Conclusions

This study is a contribution in the field of agroecology and provides information on the relationships between traditional and conservation agronomic practices on soil CO_2 emissions under a durum wheat–potato rotation system, a typical cropping system in central Italy and the whole Mediterranean area.

The effects of various tillage practices were stronger than those of fertilization sources on soil CO_2 emissions during wheat growing seasons.

Higher soil CO_2 emissions were obtained in organic fertilizer compared to those in inorganic fertilizer under the study conditions.

During wheat growing seasons, subsoiling tillage not only produces the same crop yield as conventional practices, but also increases the carbon content in the soil and requires lower CO_2 emissions for the same production.

Significant relationships between soil temperature and soil water content and soil CO_2 were detected in the study. However, these relationships were found only for durum wheat. Sustainable agronomic practices (conservative tillage and organic fertilizer) included in the study showed several benefits, including increasing soil organic carbon, decreasing the demand for chemical inputs and energy, and the promotion of crop rotation. In this context, in the study's proposed rotation system, the cropping system is favored, due to its reduced soil tillage through subsoiling and organic fertilization, which can increase the soil carbon stock in the long term. The best performances in terms of agrifoods produced per CO_2 unit were determined by soil spading and plowing and by organic fertilization. Moreover, in the wheat crop, advantages in terms of soil carbon balance (soil C input/output) are determined in dry climate conditions, but there are also good performances in terms of agrifoods produced per CO_2 unit in wet climate conditions. In contrast, in the potato crop there are no advantages in terms of soil carbon balance (soil C input/output) determined by the climate, while there are good performances in terms of agrifoods produced per CO_2 unit in dry climate conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/land12020326/s1, Table S1: The ANOVA for the effects of the soil tillage practice, fertilization source, and year on the C balance in the two experimental years of durum wheat; Table S2: The ANOVA for the effects of the soil tillage practice, fertilization source, and year on the C balance in the two experimental years of potato crop.

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Chapter 4

InnoVar field experimentation

1. Introduction

Other cultivation practices were tested in this second experimental trial. In particular, the use of different quantities of fungicides and PGR (Plant Growth Regulators), the use of organic fertilizer, and the use of irrigation were evaluated, in the context of genotype selection of durum wheat. This research activity is part of the European InnoVar Project. InnoVar, "Next-generation Variety testing for better cropping on European farmland", is a Horizon 2020 "Research and Innovation Action" Project addressing innovations in plant variety testing. The InnoVar Project aims to review and rework the registration, evaluation, and information delivery processes leading to higher-yielding varieties being grown which incorporate genetic solutions to biotic and abiotic stresses and efficiency of input use achieved through breeding. This project brings together skills and knowledge from crop science, bioinformatics, soil science, meteorology, and computer science to develop and deliver methods and tools to achieve greater efficiency in on-farm testing processes and resource use to enable the EU and its farmers to maximize the potential of their land, in terms of both yield and environmental sustainability.

The general objectives are:

1. Identify crop characteristics and sustainability criteria which indicate the capacity of varieties to maintain yield under more variable conditions and more sustainable crop management practices.
2. Develop precise, rapid and automated methods for DUS (Distinctness, Uniformity and Stability) testing in compliance with European/international requirements.
3. Revise and develop VCU (Value for Cultivation and Use) trialing processes to provide data on characters that contribute to the capacity of new varieties to maintain yield under more variable conditions and sustainable crop management practices.
4. Exploit synergies between DUS and VCU testing using genomics, phenomics, weather and soil data, and machine learning to set up databases and reference collections.
5. Apply the methods and techniques developed for wheat to other cereals and other crop types.
6. Develop new tools for the evaluation and detection of variety characteristics, using genomic, phenomic and digital technologies.

7. Analyse and review existing systems for providing and delivering information about varieties and facilitate variety specialists in adopting and developing new effective methods and tools for dissemination.

It is now known that climate change is playing a major role in the earth's ecosystems. Extreme and increasingly frequent events are threatening food security on a global scale [1]. In addition to the direct impact caused by heat and water stress, there is also the indirect impact climate change has on fertilizer supplies and the presence of pathogens and pests [2]. In addition to this, the efforts of scientific research, which are focusing on increasing agricultural yields, are failing to keep pace with the soaring population growth [3]. It is particularly feared that the yields of the world's three most important crops (*Zea mays* L. -maize-, *Oryza sativa* L. -rice-, *Triticum* L. -wheat-), which together produce 57% of the world's agricultural calories, may become stagnant or even decline [4]. Specifically, wheat production is highly sensitive to climatic and environmental variations [5]. Hence a need for new wheat cultivars better suited to future soil and climate conditions [6].

Advances in the quality of varieties available to European farmers over the past 50 years have been achieved through the DUS and VCU processes enshrined in EU directives. After successful completion of DUS and VCU, varieties are added to national lists (NL) and automatically added to the EU Common Catalogue (CC) each year. For example, traits that influence how the crop grows on the farm and how agricultural products are used are evaluated in VCU tests. These are usually complex data strongly influenced by the environment and genotype x environment (G x E) interactions. In addition, selection for pathogen resistance, an integral part of sustainable production, is also analyzed in these processes. Infections are first evaluated in VCU tests and then disease resistance/susceptibility genes are identified in DUS tests. Nevertheless, economic and environmental challenges are constantly evolving, putting pressure on both agricultural and variety evaluation systems. Variety evaluation processes have evolved in response to these changing influences and advances in science and technology. Currently, rapid developments in genomics, phenomics, molecular biology, and digital technologies are opening up a multitude of opportunities for a "next generation", but in parallel, there is a need to measure the response of crop varieties to environmental challenges and biotic and abiotic stresses, which increasingly affect crops.

With InnoVar we aim to develop tools and models that will enable evaluators to assign varieties into “High-Performance and Low-Risk” (HPLR) categories based on their performance in the field under varying input regimes and environmental conditions in the target market. This HPLR categorization is a new label being developed by InnoVar to promote an understanding of "fit-for-purpose" varieties and their performance by farmers. This new varietal categorization mark will be presented in the form of an InnoVar App for farmers to review to enable them to select varieties suited to their "growth scenarios" (climate, soil, and disease risk) with greater resilience to climate change, thus moving toward sustainability across the EU for wheat cultivation. The 4.5-year Project, which started in 2020, is based on historical data that have been enriched with new morphological, phenomic, genomic, soil and meteorological data and, thanks to machine learning, the traits that can be used to classify wheat varieties (*Triticum durum* and *aestivum*) in terms of sustainability and resilience to biotic and abiotic stresses. The robustness of the methods and tools has been tested and validated in a wide range of experiments involving 18 European partners, represented by 11 locations which are divided into five agroclimatic zones. The series of experiments focused on the application of fungicides and growth regulators, to drought, irrigation, and organic conditions. Yield penalty, as a sustainability measure, has been used both to identify variety traits that are advantageous for these different growing scenarios and to provide valuable information to farmers in their decision-making. The aim was to evaluate the performance of varieties and their characters with different management approaches. In particular, performance was evaluated under two different management regimes relating to fungicides and growth regulators (PGR), called Full and Minimal, and under an Organic approach and an Irrigation regime.

2. Materials and Methods

2.1 Site Description

The experimental field is located in the Experimental Farm of the University of Tuscia (Viterbo), in central Italy, with coordinates 42°42′ N, 12°07′ E, and an altitude of 310 m above sea level. The Project selected the experimental sites based on the agroclimatic zones representing the European territory and the type of soil. Among the three different agroclimatic zones (ACZ), Pannonian, Mediterranean, and Maritime South, the Unitus experimental site falls into the latter ACZ. In the experimental years examined (2021/2022, 2022/2023), the data were collected by the farm's internal meteorological station which recorded the following data represented in Figure 1.

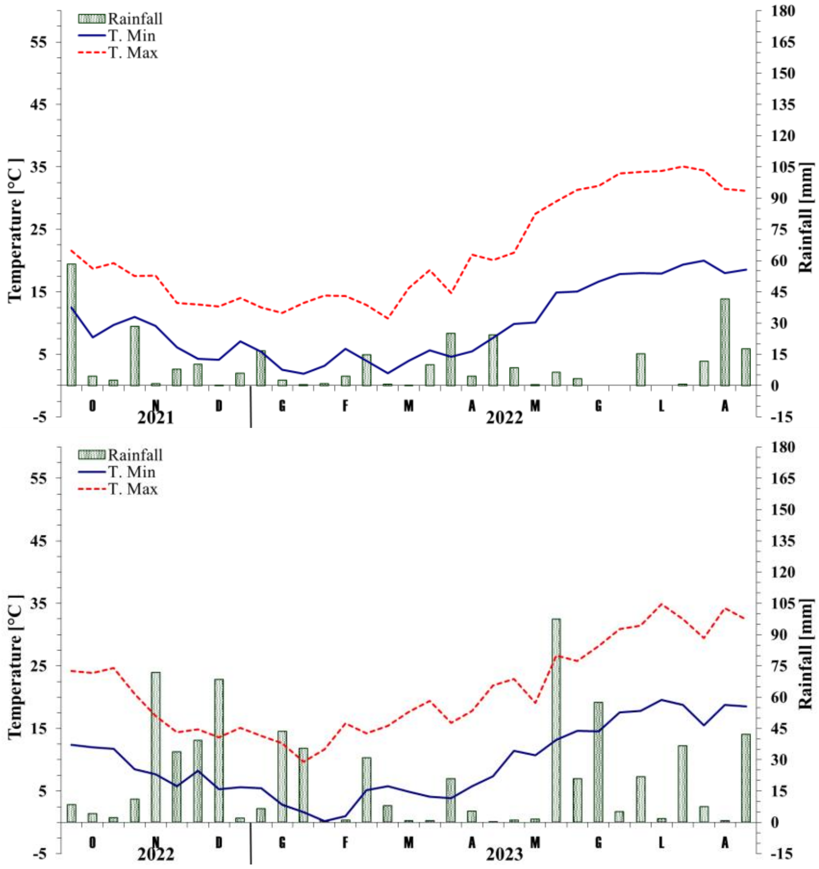


Figure 1. Weather data at 10-day intervals (maximum (red line), minimum (blue line) temperatures (°C) and rainfall (columns, mm)) at the experimental site during the experimental period in 2020/2021 and 2021/2022 growing seasons.

The soil of the experimental site is classified as Cambisols and has the following characteristics reported in Table 1.

Table 1. Soil characteristics of the experimental field.

	Available Water Capacity (AWC) [vol.] 0-20cm	Soluble Phosphorous (P) [mg/kg] 0-20cm	Extractable Potassium (K) [mg/kg] 0-20cm	pH-CaCl2 0-20cm	CaCO3 [g/kg] 0-20cm	C:N ratio 0-20cm
Cambisols	0,11	21,46	689	7,03	113,23	9,71

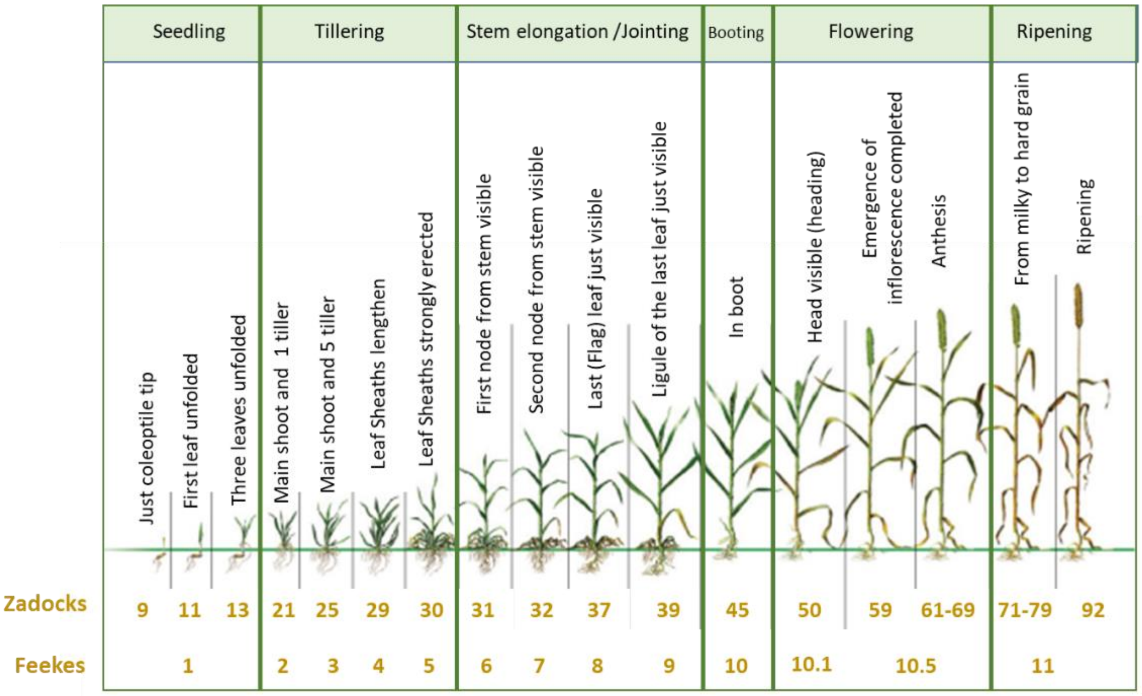
2.2 Experimental Details

The study was conducted to evaluate the possible effects on the yield and quality of 15 selected durum wheat varieties under 4 different treatments. The InnoVar protocol, based on the Zadocks scale (Figure 2), involved the following treatments.

- Full: two fungicide applications, one at growth stage (GS) 33-37 with Fungicide Seguris-Xtra® and PGRs Moddus® (dose 1 l ha^{-1}) and a second at 45-51 GS with Fungicide Prosaro® (dose 1 l ha^{-1});

- Seguris- Xtra®: pure isopyrazam 9.0 g (100 g/l)
  pure azoxystrobin 9.0 g (100 g/l)
  pure cyproconazole 7.2 g (80 g/l)
  conformulants q.b. to 100 g
- PGRs Moddus®: trinexapac-ethyl (250 g/l)
- Prosaro®: pure prothioconazole 12.7 g (125 g/l)
  pure tebuconazole 12.7 g (125 g/l)
  co-formulants q.b. to 100 g
  contains N, N-Dimethyldecane-1-amide

- Irrigated: same treatment as Full and irrigation from about mid-March until a few weeks before harvest;
- Minimal: one fungicide application in GS 45-51 with Fungicide Prosaro® (dose 1 l ha^{-1});
- Organic: no treatment.
- In all treatments except Organic, Granstar ultra sx® weed control was applied in April at a dose of 40 g ha^{-1}. All agronomic practices (tillage and fertilization) were carried out according to the field common practices applied in the area (Simple Superphosphate before sowing -90 kg ha^{-1}-, Ammonium Nitrate during tillering -250 g/plot- and Urea 43% during stem-elongation -150 g/plot-; only poultry manure for the Organic field -500 kg ha^{-1}-). In both growing seasons, sowing took place in November, with a sowing rate of 420 g per plot (10 m^2). Each InnoVar VCU trial comprised three set of varieties: the first set was common to all trials, the second was common for each ACZ and the last one was site-specific. For this experimentation we analyzed a total of 15 modern varieties, belonging to the 3 categories (Table 2). The experiment was laid out in randomized block design with three replications of each treatment.



	SEEDLING GROWTH		**BOOTING**
10	first leaf through coleoptile	**41**	flag leaf sheath extending
11	first leaf unfolded	**43**	boots just visibly swollen
12	2 leaves unfolded	**45**	boots swollen
13	3 leaves unfolded	**47**	flag leaf sheath opening
14	4 leaves unfolded	**49**	first awns visible
15	5 leaves unfolded		**INFLORESCENCE (EAR EMERGENCE)**
16	6 leaves unfolded	**51**	First spikelet of inflorescence just visible
17	6 leaves unfolded	**52**	¼ of inflorescence emerged
18	8 leaves unfolded	**55**	½ of inflorescence emerged
19	9 or more leaves unfolded	**57**	¾ of inflorescence emerged
	TILLERING	**59**	inflorescence completed
20	main shoot only		**ANTHESIS**
21	main shoot and 1 tiller	**60**	beginning of anthesis
22	main shoot and 2 tillers	**64**	anthesis half-way
23	main shoot and 3 tillers	**68**	anthesis completed
24	main shoot and 4 tillers		**MILK DEVELOPMENT**
25	main shoot and 5 tillers	**71**	caryopsis watery ripe
26	main shoot and 6 tillers	**73**	early milk
27	main shoot and 7 tillers	**75**	medium milk
28	main shoot and 8 tillers	**77**	late milk
29	main shoot and 9 or more tillers		**DOUGH DEVELOPMENT**
	STEM ELONGATION	**83**	early dough
30	Ear at 1 cm	**85**	soft dough
31	1st node detectable	**87**	hard dough
32	2nd node detectable		**RIPENING**
33	3rd node detectable	**91**	caryopsis hard (difficult to divide by thumb-nail)
34	4th node detectable	**92**	caryopsis hard (can no longer be dented by thumb-nail)
35	5th node detectable	**93**	caryopsis loosening in daytime
36	6th node detectable		
37	flag leaf just visible		
39	flag leaf ligule/collar just visible		

Figure 2. Zadocks' scale and details of the stages of the wheat cycle.

Table 2. List of the varieties of durum wheat

Variety	Breeding Company	Category
Antalis	Limagrain	Control
Claudio	SIS (Società Italiana Sementi)	Control
Don-Ricardo	Adaglio Sementi	Control
Dylan	Società Produttori Sementi S.p.a	Site specific
Furio-Camillo	SIS (Società Italiana Sementi)	Site specific
Heraklion	Società Produttori Sementi S.p.a	Site specific
Iride	PSB (Società Produttori Sementi Bologna)	Control
Marakas	Ragt Italia	Site specific
Marco-Aurelio	SIS (Società Italiana Sementi)	ACZ
Monastir	Ragt Italia	Control
Odisseo	PSB (Società Produttori Sementi Bologna)	ACZ
Saragolla	PSB (Società Produttori Sementi Bologna)	ACZ
Simeto	Po.Se.Me.	ACZ
Svevo	PSB (Società Produttori Sementi Bologna)	ACZ
Tirex	Co.Na.Se.	Site specific

2.3 Data Collection

During the two growing seasons, data were collected relating to any pathogen attacks in particular, the presence of the main problems relating to wheat was verified, such as Yellow Rust (*Puccinia striiformis* f.sp. *tritici*), Brown Rust (*Puccinia triticina*), Septoria (*Septoria tritici*) and Fusarium ear (*Fusarium* spp.) and their presence has been reported visually if the % of infection of the plant in the plot was > 40%. Plant height was measured at maturity from the soil surface up to the tip of the spike, excluding awns. At the time of plot harvest, the yield of each test was determined, and quality measurements were performed on a sub-sample of 100 g of dry grain with a handheld NIR analyzer (Aurora® GraiNit S.r.l) (Figure 3).

Figure 3. Handheld NIR analyzer (Aurora® GraiNit S.r.l).

2.4 *Statistical Analysis*

Full factorial analysis of variance (4 treatments x 2 years x 15 varieties) was applied to assess the effects of treatments on grain yield and quality. Means were compared adopting the Tukey's HSD (Honestly Significant Difference) at $p < 0.05$. The variance analysis of field data was carried out using the statistical software JMP package 4.0 (JMP Statistical Discovery, Cary, NC, USA).

3. Results and Discussion

Climatic conditions, mainly linked to rainfall, have varied over the years. The 2021/2022 growing season was found to be extremely dry with total rainfall during the October-August period of 329.4 mm. While, in the 2022/2023 growing season, 690.6 mm of rain fell in the same period. In the 2022/2023 season, there were two peaks of 100.0 mm and 83.4 mm respectively in May and June whereas in 2021/2022 in the same months rainfall of 15.4 mm and 3.4 mm were recorded. Air temperature follows the long-term average trend and gradually decreases after sowing durum wheat until February, and then gradually increases, reaching the highest temperature before harvesting wheat seeds. Mean air temperature was similar between growing seasons (13°C).

Table 3 shows the results of the analysis of variance relating to productivity, plant height, and quality values.

Table 3. Analysis of the Variance relative to the productive and qualitative parameters of durum wheat.

	Prob > F							
	DF	Yield	HI	Plant height	Protein	Protein/ Yield	Starch	Fat
Variety	14	0.0215*	<.0001*	<.0001*	0.0082*	0.3375	0.0035*	0.0291*
Treatment	3	0.1534	<.0001*	<.0001*	<.0001*	0.0660	0.3444	0.4693
Variety*Treatment	42	0.9484	0.3037	0.7417	0.0019*	0.5213	0.1944	0.2804
Year	1	0.4033	0.0135*	0.0160*	<.0001*	0.8389	<.0001*	<.0001*
Variety*Year	14	0.2132	0.0006*	0.7026	0.0070*	0.5903	0.0212*	0.0299*
Treatment*Year	3	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.1509

The results show that Grain Production was influenced by Variety and Treatment x Year interaction. Averaged over years (Table 4), the most productive variety was Heraklion, with a total of 3670.76 kg ha^{-1} of dry Grain Yield, and the variety with the lowest production was Simeto (2568.13 kg ha^{-1}). This result can be explained by the typical characteristics of the genotype, but also by the fact that the first variety belongs to the "Site-specific" (SP) category and is therefore already adapted to the environment in which it was tested, while the second variety belongs to the category “Agro-Climatic Zone” therefore not strictly linked to the Upper Lazio site. Within the SP category Heraklion is confirmed as the most productive variety, while Furio-Camillo has the lower average production of 2884.84 kg ha^{-1}, but not significantly different from other varieties of the same category. In the "Control" category (C) it was the Monastir variety that produced the most with an average value of 3515.19 kg ha^{-1}, followed by Claudio (3219.33 kg ha^{-1}). Finally, in the ACZ category, the varieties were all statistically equal except for the Simeto variety which turned out to be the least productive.

Table 4. Effect of the variety (V) factor on durum wheat production data. Values followed by the same lower-case letters are not different according to Tukey’s HSD (<0.05).

			Yield [kg ha^{-1} DM]		HI		Plant height [cm]	
V	Antalis	Control	2832,98	ab	0,35	ac	70,15	ac
	Claudio	Control	3219,33	ab	0,35	ac	75,33	ab
	Don-Ricardo	Control	2695,25	ab	0,33	ac	71,12	ab
	Dylan	Site specific	3084,13	ab	0,33	ac	70,36	ab
	Furio-Camillo	Site specific	2884,84	ab	0,29	c	71,55	ab
	Heraklion	Site specific	3670,76	a	0,38	ab	72,20	ab
	Iride	Control	2958,27	ab	0,39	a	62,11	c
	Marakas	Site specific	3512,83	ab	0,37	ab	69,87	ac
	Marco-Aurelio	ACZ	3017,11	ab	0,37	ab	72,43	ab
	Monastir	Control	3515,19	ab	0,37	ab	77,76	a
	Odisseo	ACZ	3040,36	ab	0,36	ac	71,69	ab
	Saragolla	ACZ	2864,68	ab	0,35	ac	71,54	ab
	Simeto	ACZ	2568,13	b	0,29	c	69,44	bc
	Svevo	ACZ	3035,00	ab	0,32	bc	74,08	ab
	Tirex	Site specific	3048,33	ab	0,34	ac	72,08	ab

Studying genotype-environment interactions is important for evaluating the adaptation of varieties, if the classification of genotypes differs between environments, this makes it more difficult to identify superior reproductive genotypes since measured trait values may be influenced more by environmental variation than by genotype [7]. As regards the Harvest Index, the ratio of grain yield to total biomass, the average value of all the varieties studied was 0.35, with a maximum value of 0.39 for the Iride variety and a minimum value of 0.29 for the Furio-Camillo and Simeto varieties. Category C had an average HI of 0.35, category SP 0.34, and 0.33 for category ACZ. The average Height of the culms was around 71.45 cm. The tallest variety was Monastir, with a Height of 77.76 cm and the shortest was Iride with 62.11 cm of Height. Table 5 shows the results of the effects of the Treatment x Year interaction on Yield, HI, and Plant Height. In general, the drier year 2022 produced an average of 2836.47 kg ha^{-1}, while 2023, where constant rainfall was recorded throughout the season and peaks in the growing season, produced 3293.80 kg ha^{-1} on average.

Table 5. The interaction effect of the treatment (T) x year (Y) on durum wheat production data. Values followed by the same lower-case letters are not different according to Tukey's HSD (<0.05).

		Yield [kg ha^{-1} DM]				HI				Plant height [cm]			
		2022		2023		2022		2023		2022		2023	
TxY	Full	2231,10	ce	4278,09	ab	0,38	ab	0,32	ab	55,62	f	81,12	bc
	Irrigated	4080,16	ac	1933,01	e	0,43	a	0,32	ab	64,85	ce	64,01	df
	Minimal	2308,65	ce	3917,30	ac	0,38	ab	0,31	b	57,75	f	81,39	bc
	Organic	2726,00	ce	3046,82	bd	0,39	ab	0,23	c	78,54	ad	88,27	a

As expected, Grain Yields were significantly higher in the season with higher rainfall, in which the total amount of precipitation during the vegetative stages was also higher, and this may have led to an extension of the vegetative phase, expressed with a greater number of tillers [8]. Specifically, the interaction that obtained the highest production was the Full-2023 interaction, with an average of 4278.09 kg ha^{-1}, while the interaction that produced the least was the Irrigated-2023, probably because the supply of water combined with the abundant rain was able to leach the nitrates, creating conditions of nutritional insufficiency [9]. It is interesting to note that in both years the productions in Full and Minimal did not differ statistically. The results relating to the Harvest Index follow the same trend as the yield during the year 2022, with the highest value for the Irrigated (0.43 HI), followed by the Organic (0.39), Full (0.38) and Minimal (0.38) which have equaled statistically. However, in 2023 the highest HI value was found to be, in the Full treatment (0.32) and the Irrigated one (0.32) and followed by the Minimal (0.31) and finally by the Organic (0.23). These data could be explained by the significance found between Treatments and Year regarding plant height. The average height of the plants in the various treatments in 2022 was 64.19 cm, while in 2023 it was 78.69 cm. Here too, the difference in growth can be explained by the different climatic trends in the seasons. It is known that plant height is usually negatively correlated with the Harvest Index [10], and that increases in yield, in terms of biomass production and allocation, are due to changes in the distribution of photosynthates within the plant, that is, to improvements in HI in association with the decrease in plant height [11].

Regarding findings relating to pathogen attacks, it was observed, and confirmed by the other VCU tests of the InnoVar Project, that disease pressure was very low. Among all the diseases taken into consideration by the protocol (Yellow Rust, Brown Rust, Septoria, and Fusarium), in the varieties analyzed, only the presence of Fusarium was found to be higher than 40 % (minimum detection limit). The percentage represents the quantity of infected plants within the plot. In detail (Table 6), it can be noted that this pathogen was also not very present in the tests and, above all, it did not attack all the varieties. Moreover, the attack had no significant impact on the quality and quantity of the grain. The varieties that were most affected were Antalis and Saragolla. In particular, Antalis reported Fusarium attacks in all trials, regardless of treatment. The difference in the varieties can be explained by the different tolerance characteristics of each genotype. It is known that the Antalis variety is "moderately resistant" to fusarium disease [12]. It is interesting to note how the attacks (above 40 %) only occurred in 2023, the rainiest year. It is proven that high rainfall and relative humidity are crucial meteorological factors for the infection of wheat grain by Fusarium [13]. This also proves the fact that among the treatments, it was the Irrigated treatment that presented more attacks. However, there were no significant differences between the Full and Minimal treatments. This means that, in areas such as central Italy, where the pressure from pathogens is reduced, even in rainier years, it is possible to reduce the dose of fungicide and biostimulants.

Table 6. Presence plant per plot (in %) of Fusarium attack.

			Fusarium [%]											
			Full			Irrigated			Minimal			Organic		
			R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Antalis	Control	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	50	80	90	-	50	-	-	-	70	60
Claudio	Control	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	50	-	-	-	-	-	-	-	-
Don-Ricardo	Control	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	40	-	-	-	-	-	-	-	80
Dylan	Site specific	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	90	-	-	-	-	-	-	-	-
Furio-Camillo	Site specific	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	-	40	-	-	-	-	-	-	-
Heraklion	Site specific	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	-	-	-	-	-	-	-	-	-
Iride	Control	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	-	-	-	-	-	-	-	-	-
Marakas	Site specific	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	-	-	-	-	-	-	-	-	-
Marco-Aurelio	ACZ	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	40	-	80	-	-	-	-	-	-
Monastir	Control	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	-	-	-	-	-	-	-	-	-
Odisseo	ACZ	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	-	-	-	-	-	-	-	-	-
Saragolla	ACZ	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	50	-	-	60	-	-	-	-	90	-
Simeto	ACZ	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	60	-	-	-	60	-	-	-	-
Svevo	ACZ	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	-	-	-	-	-	-	-	-	-
Tirex	Site specific	2022	-	-	-	-	-	-	-	-	-	-	-	-
		2023	-	-	-	-	-	-	-	-	-	-	-	-

In this study, following Near-Infrared Reflectance Spectroscopy measurements, Proteins reported significance between all factors and their interactions, Lipids (fat) were found to be significantly conditioned by Varieties, from the Year and their interaction and Starch, also reported significance for Varieties, the Year, Variety x Year and the interaction between Treatment and Year (Table 3). The results for Proteins are shown in Figure 4.

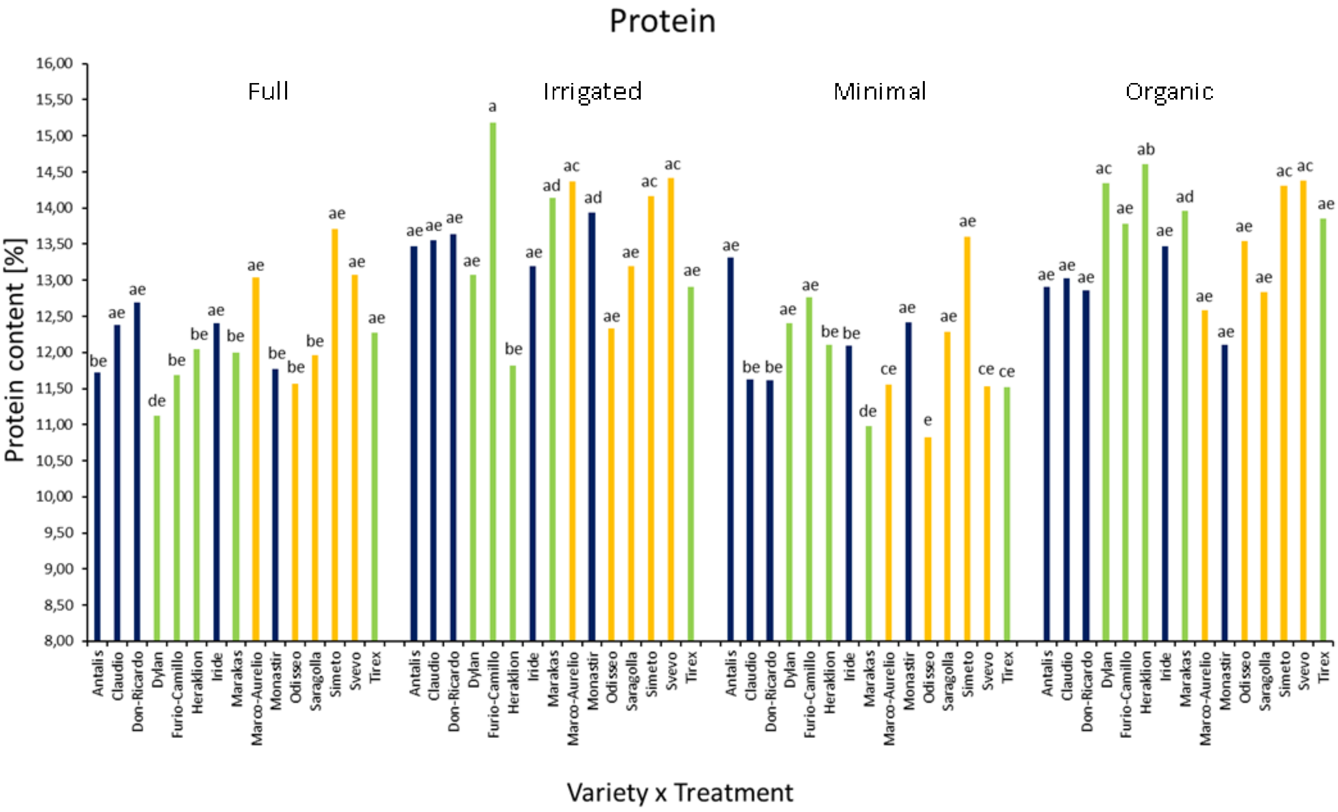


Figure 4. The interaction effect on protein content. Values followed by the same letter are not significantly different according to Tukey's HSD (<0.05). Blue: Control, yellow: ACZ, green: Site specific.

The figure shows how the 15 varieties reported significantly different values in the four treatments. Protein values (CP) ranged from 10.83% to 15.19%, complying with standard values. In the Variety x Treatment interaction, treatments that found higher percentages in protein content were the Irrigated treatment and the Organic that did not show significant differences between them, while they obtained lower performances and were not significantly different from each other in the Full and Minimal treatments. In the higher protein content range, we find varieties such as Furio-Camillo (15,19% CP in Irrigated and 13,78 % in Organic), Svevo (14,42% CP in Irrigated and 14,38% in Organic), Simeto (14,31% CP in Organic and 14,17% in Irrigated) and Marakas (14.14% CP in Irrigated and 13.96% in Organic) which have benefited positively from Irrigated and Organic treatments. Among the varieties with lower protein content is Dylan in Full treatment (11,12 % CP), Marakas in Minimal treatment (10,98 % CP), and Odisseo in Minimal treatment with 10,83% CP. It is known that the protein content is influenced by the genotype, but also by the environment [14]. The genotypic variation of protein content can be attributed to differences in the absorption of nitrogen from the soil before the anthesis, in the activity of the root system during the filling of grains, the translocation efficiency of nitrogenous substances from vegetative tissues to grains, and in the harvest index [15]. Conflicting results were obtained about the relationship between protein in the grain and water stress. The present trial found higher protein values in the varieties associated with the Irrigated and Organic treatments, and in the interaction between Treatment x Year, higher values were obtained in the Irrigated and Organic treatments in the year 2022 (Figure 5). Many authors agree that an average increase in protein content is reported in water-deficit conditions [16]. This is confirmed also in the year factor, where 2022, much drier, has increased the value of protein content in the grain. However, to explain the best results in the Irrigated treatment, it can be specified that it is the timing of the event of water stress to more influences the duration of the grain filling and therefore the final protein composition [17,18]. As can be seen, the 2021-2022 season reported very strong stress in the May-June period (grain-filling period) and here irrigation probably supported the crop but was unable to overcome the strong stress which therefore is converted into a higher concentration of proteins. The results obtained for the treatment in Organic-2022 could be due to better microbial activity in this field which, despite the drought, managed to maintain an adequate nitrogen content for the crop. It is known that greater nitrogen availability increases the protein content of wheat grain [19]. On the contrary, treatments of fungicide and biostimulants in Full and Minimal, did not show significant differences. The results relating to the protein content/production ratio are in accordance with the results of Jenner *et al*. [20] and Mariem *et al*. [21] who say that the increase in wheat yield has often been associated with quality losses of wheat. In general, 2022 reported lower wheat production and a higher protein content, vice versa, 2023 was more productive, but with lower CP values (Figure 6). Figure 7 shows the significance between factors Treatment and Year for Protein Yield. The highest ratio was obtained for the treatment Irrigated in 2022 (0.59 t ha^{-1}) and the lowest for the treatment Irrigated 2023 (0.23 t ha^{-1})

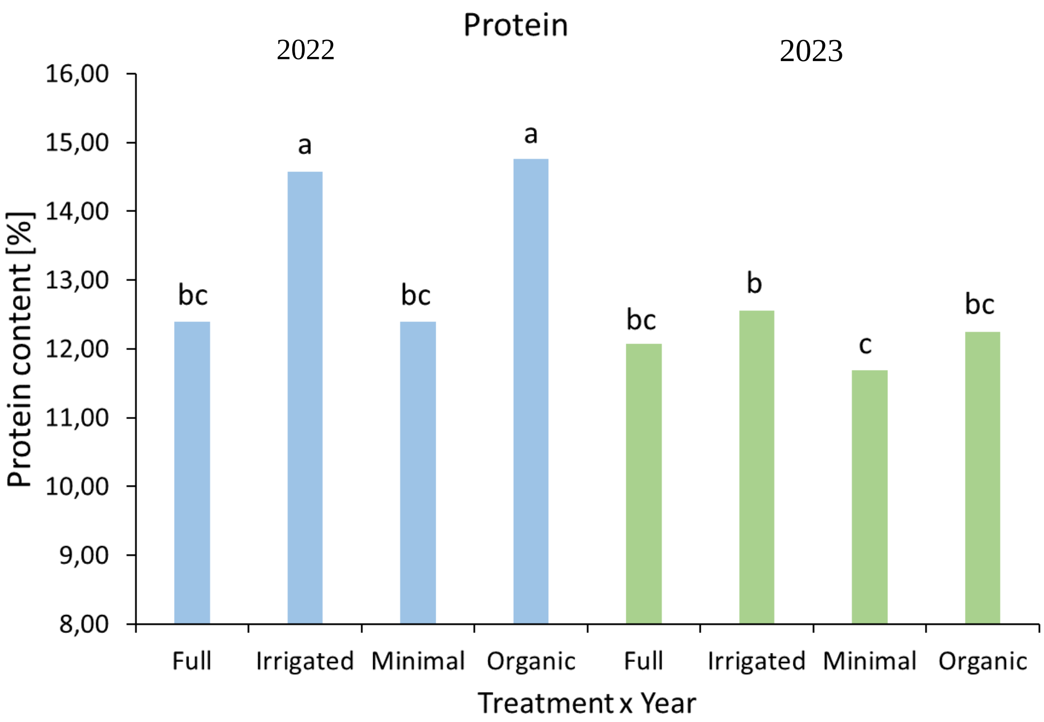


Figure 5. The interaction effect of treatment x year on protein content. Values followed by the same letter are not significantly different according to Tukey's HSD (<0.05). Light blue: 2022, green: 2023.

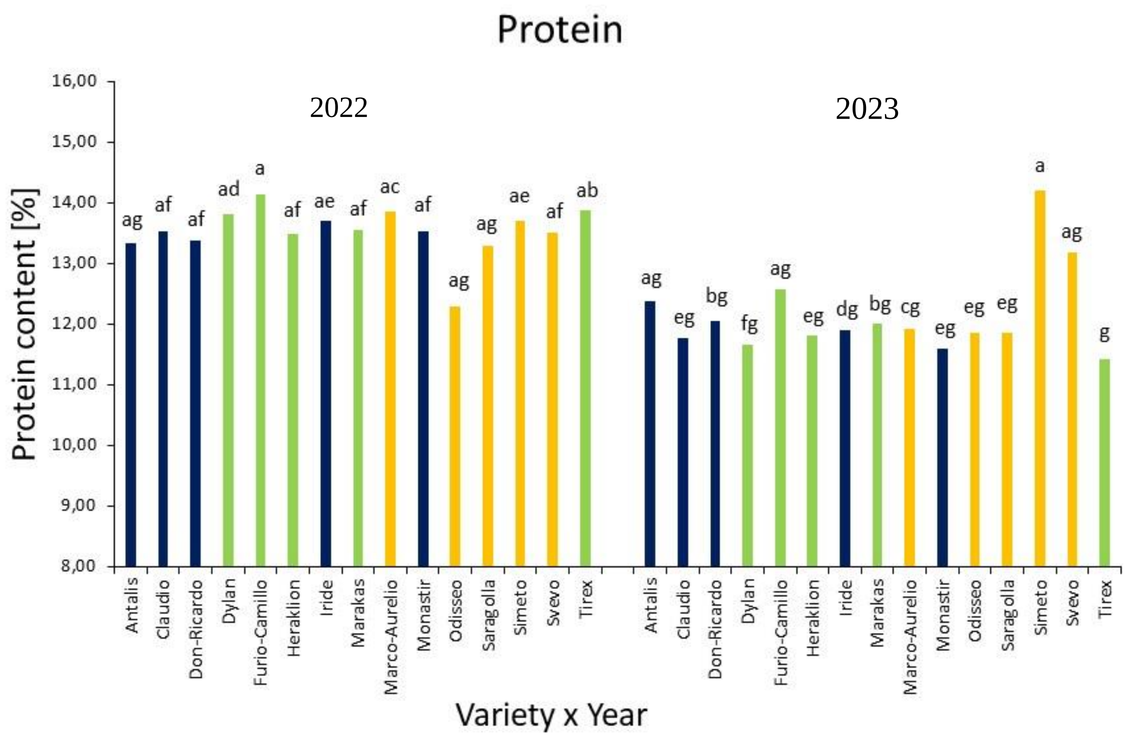


Figure 6. The interaction effect of variety x year on protein content. Values followed by the same letter are not significantly different according to Tukey's HSD (<0.05). Blue: Control, yellow: ACZ, green: Site specific.

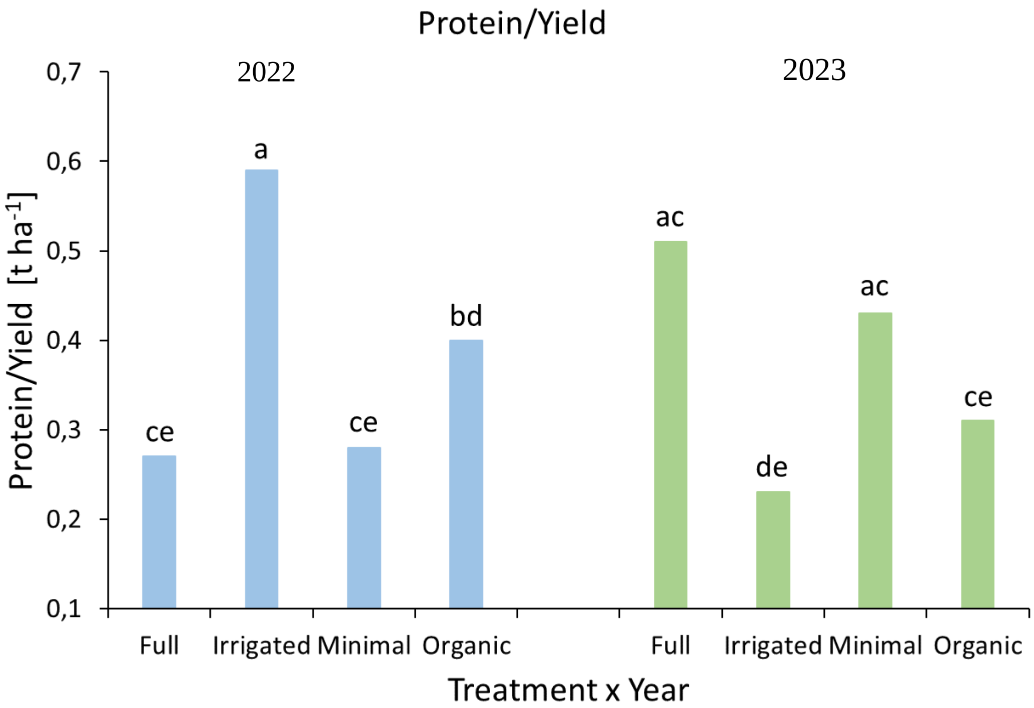


Figure 7. The interaction effect of treatment x year on protein content/yield. Values followed by the same letter are not significantly different according to Tukey's HSD (<0.05). Blue: Control, yellow: ACZ, green: Site specific.

The amount of Starch was also statistically different in the two growing seasons (Figure 8). 2022 reported an average Starch value of 58.88% and 2023 of 57.08%. In addition, the highest values were obtained for the treatments Irrigated and Organic-2022 and the variety Odisseo-2022 with a value of 59.56% Starch (Figure 9). Carbohydrate accumulation dynamics in durum wheat are well documented, however, most studies report that water stress during the grain-filling phase reduces starch accumulation [22]. Nevertheless, higher starch values in durum wheat grains under water stress were also obtained in other Italian localities by Graziano *et al*. [23].

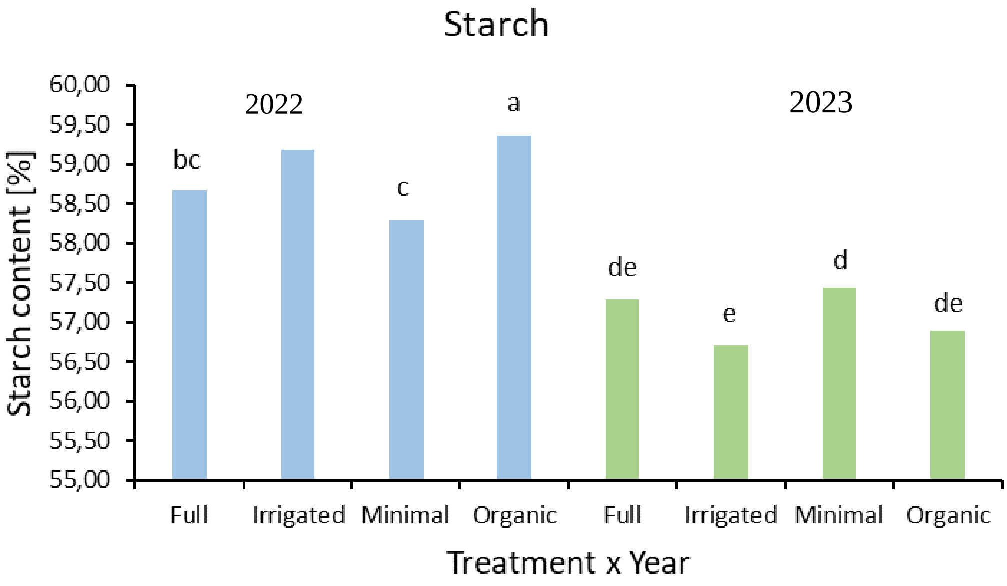


Figure 8. The interaction effect of treatment x year on starch content. Values followed by the same letter are not significantly different according to Tukey's HSD (<0.05). Light blue: 2022, green: 2023

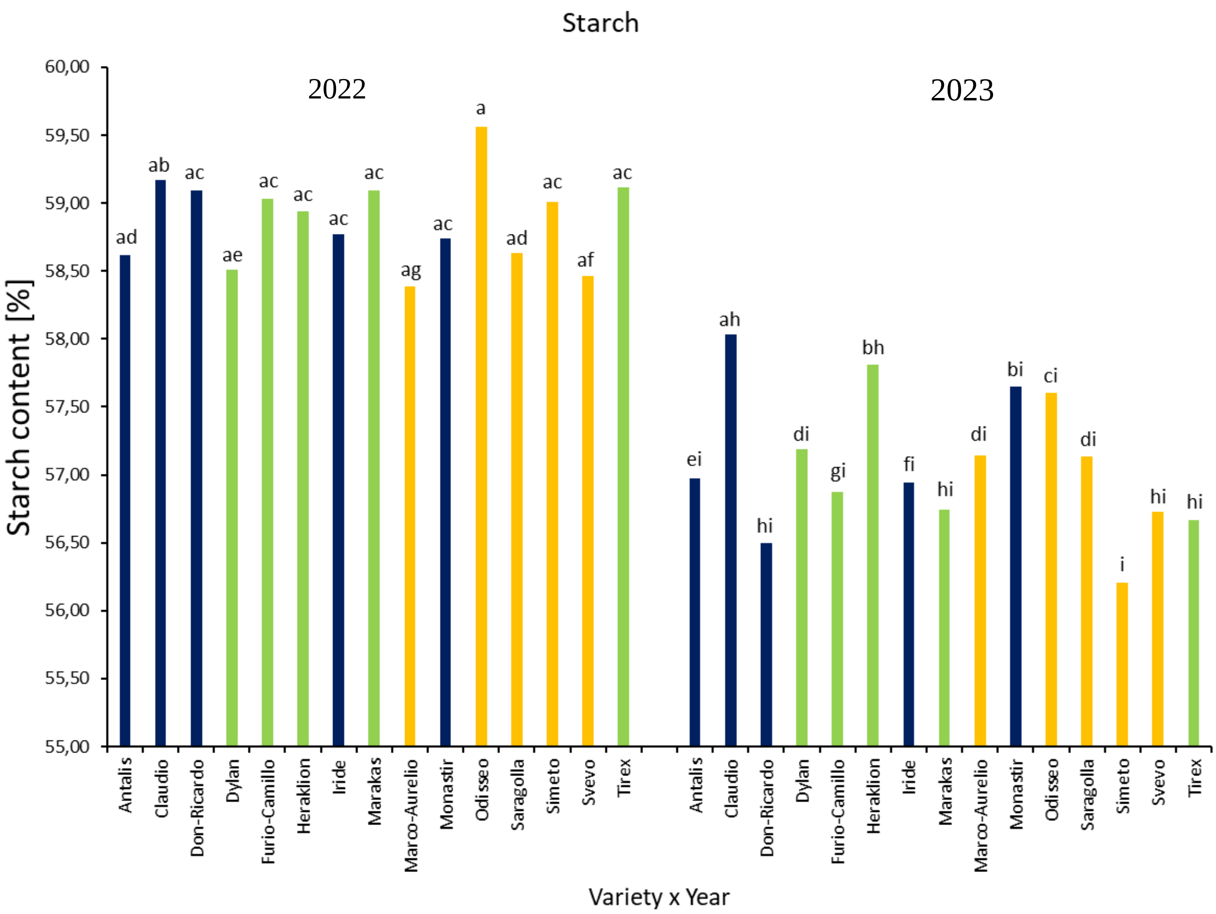


Figure 9. The interaction effect of variety x year on starch content. Values followed by the same letter are not significantly different according to Tukey's HSD (<0.05). Blue: Control, yellow: ACZ, green: Site specific.

The Lipid content in the wheat grain depends on several factors, some of which are genetic, such as species and varieties, and others depend on the environment and are linked to soil and climatic conditions and agricultural practices [24,25]. Lipids ranged from 1.47% to 1.64% (Figure 10). Higher values were obtained in 2023 with an average of 1.61% compared to the average value of 1.50% in 2022. The varieties with the highest Lipid content are all in interaction with the year 2023, Saragolla and Don-Ricardo with 1.64%, Simeto with 1.63%, and Svevo, Antalis, and Iride with 1.62% lipids.

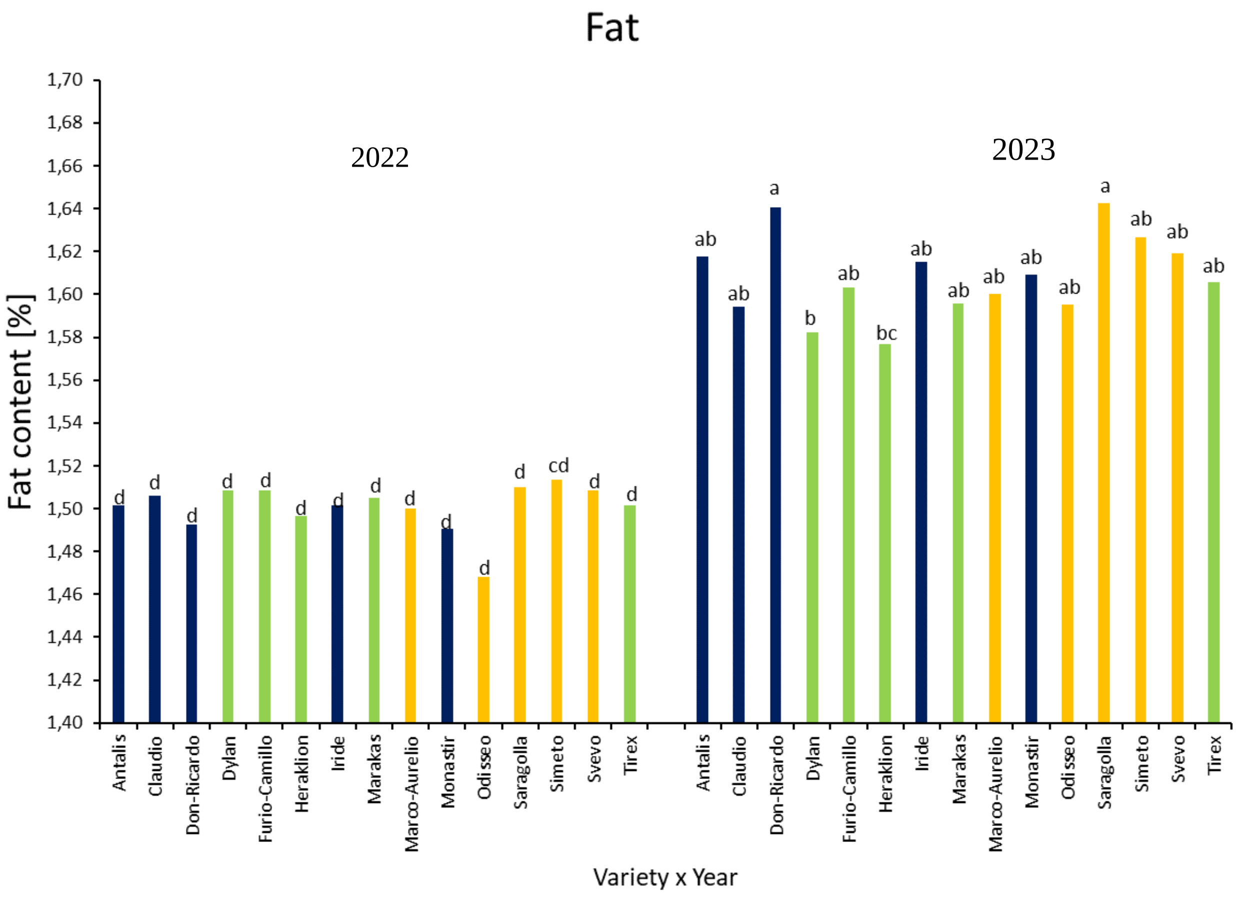
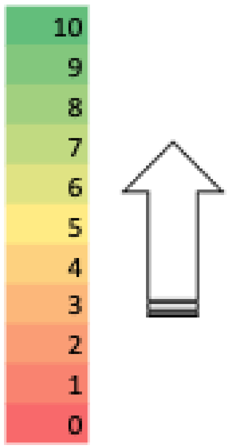


Figure 10. The interaction effect of variety x year on fat content. Values followed by the same letter are not significantly different according to Tukey's HSD (<0.05). Blue: Control, yellow: ACZ, green: Site specific.

From the "heat map" in Table 7, we can see that there is a negative relationship between the production and content of proteins and starch and a positive relationship between yield and fat. In the year 2022 there was an average production of 2.84 t ha^{-1} and an average of 13.40 % protein content, 58.67 % starch and 1.51 % lipids. In 2023, an average production of 3.30 t ha^{-1} was reached, with an average of protein content of 12.13 % and 57.22 % of starch and 1.60 % of lipids. Among the varieties that, on average, performed worse in terms of production and quality, we find Simeto-23 with a production of 2.44 t ha^{-1} and 12,57 % of CP, 56.87 % of starch and 1.60 % of fat. Among the best there is Svevo-23, variety of ACZ category, with 3.48 t ha^{-1} of yield, 13.19 % of CP, 56.73 % of starch and 1.62 % of fat.

Table 7. Heat map. The color gradation indicates increasing gradients from red to green.

Variety x Year	Yield [t ha^{-1}]	Protein [%]	Starch [%]	Fat [%]
Furio-Camillo-22	2,19	14,13	59,03	1,51
Simeto-23	2,44	12,57	56,87	1,60
Marco-Aurelio-22	2,56	13,85	58,39	1,50
Don-Ricardo-22	2,71	13,37	59,10	1,49
Svevo-22	2,71	13,51	58,46	1,51
Dylan-22	2,72	13,82	58,51	1,51
Antalis-23	2,76	12,37	56,97	1,62
Iride-22	2,76	13,70	58,77	1,50
Tirex-22	2,76	13,87	59,12	1,50
Odisseo-22	2,78	12,28	59,56	1,47
Don-Ricardo-23	2,79	12,04	56,50	1,64
Simeto-22	2,82	13,70	59,01	1,51
Saragolla-22	2,84	11,65	57,19	1,58
Marakas-22	2,99	12,37	56,97	1,62
Antalis-22	3,00	14,13	59,03	1,51
Saragolla-23	3,10	13,29	58,63	1,51
Claudio-22	3,14	13,53	59,17	1,51
Monastir-22	3,16	13,53	58,74	1,49
Iride-23	3,26	11,89	56,94	1,62
Claudio-23	3,29	11,76	58,03	1,59
Odisseo-23	3,39	11,86	57,61	1,60
Heraklion-22	3,41	13,49	58,94	1,50
Tirex-23	3,44	11,41	56,67	1,61
Svevo-23	3,48	13,19	56,73	1,62
Dylan-23	3,57	11,65	57,19	1,58
Marco-Aurelio-23	3,64	11,92	57,14	1,60
Furio-Camillo-23	3,68	12,57	56,87	1,60
Monastir-23	3,87	11,58	57,65	1,61
Heraklion-23	3,93	11,80	57,81	1,58
Marakas-23	4,03	12,00	56,74	1,60
Tot. Average	**3,11**	**12,83**	**57,98**	**1,55**
Average 2022	**2,84**	**13,40**	**58,67**	**1,51**
Average 2023	**3,38**	**12,13**	**57,22**	**1,60**



4. Conclusions

Durum wheat is a well-adapted species to the Mediterranean climate; however, different climatic seasons significantly affected all qualitative and quantitative characteristics of durum wheat. Significant differences were found among varieties and in the interaction between treatment x year in production components. As expected, Grain Yields were significantly higher in the season with higher rainfall (2023). The interaction that obtained the highest production was the Full-2023 interaction, with an average of 4278.09 kg ha-1, but it is interesting to note in 2023 the productions in Full and Minimal did not differ statistically. Plant height followed the same trend, higher in the rainy season of 2023 and lower in 2022. Harvest Index, on the other hand, being negatively correlated with height was found to be higher in 2022. The only disease detected with a percentage higher than 40 % was fusarium, with attacks only in 2023, particularly in the Irrigated treatment. The different doses of fungicide and PGR expressed in the two treatments Full and Minimal did not report significant differences. Significant differences between varieties were found in all three nutritional values measured in this study. The highest protein contents were found when higher water stress occurred during grain filling (year 2022), however, higher values were found in Irrigated and Organic treatments, while fungicide treatments showed no significant differences. Starch content showed the same trend as protein content, while lipids showed a positive relationship with grain yield. Higher grain and fat production was obtained in 2023 (wettest) than in 2022. The variety that on average performed best in terms of quality quantity was Svevo in the year 2023, a variety belonging to the Maritime South agroclimatic zone.

In general, organic treatment has always shown good value in terms of quality and quantity. While fungicide and PGR treatments have had little impact on grain quality and quantity. Therefore, their use in the Mediterranean area can be reduced for the benefit of greater economic savings and environmental sustainability. The results of this study showed that in Central Italy, durum wheat production and quality and nutritional characteristics are generally sensitive to thermo-precipitative variations but are not excessively affected by pathogen attack. Together with the other data from the InnoVar Project on the influence of agronomic treatments and thermo-pluviometrical regimes on durum wheat, with a multidisciplinary approach, it will be possible to obtain high-quality durum wheat varieties suitable for the upcoming environmental constraints.

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General Conclusions

In the coming years, the reduction of natural resources will have a strong influence on the agricultural sector. The sustainable approach, understood as environmental, economic, and social sustainability, seems to be a solution for the agriculture of the future. This will have to produce enough for a growing population and will have to be resilient enough to deal with extreme events caused by climate change. One of the goals of modern agricultural research is to increase yields by decreasing inputs into the agroecosystem.

This thesis focused on the study and evaluation of sustainable agricultural systems, with particular attention to durum wheat cultivation and the wheat-potato rotation system. The research evaluated the impact on crop production and quality, soil fertility and components, and system biodiversity represented by weed communities. The evaluation occurred through different management, different levels of agrochemical and fertilizer inputs, and water regimes.

Specifically, the results obtained demonstrated that conservative practices and organic fertilization negatively interfered with the production of durum wheat grain. However, the adoption of these practices has proven to be more favorable for increasing the composition of weed species, which are more balanced in terms of competition with the crop and are more likely to preserve the biodiversity of the agroecosystem. In particular, the adoption of the subsoiler highlighted the maximum diversity of the weed flora, even if the process that seemed to be the right compromise between satisfactory wheat production and a balanced floristic composition turned out to be digging. Furthermore, this could be associated with the adoption of organic fertilization which, despite being less efficient than mineral fertilization for crops, gradually releasing nitrogen during the mineralization process, avoids the specialization of nitrophilous plants and allows for greater floristic diversity. Indeed, results obtained from potato crop studies have shown that organic fertilization can change the intensity of microbial quotient and soil-specific enzyme activity, representing a key factor in soil health. A difference in soil nutrient availability was also found in tillage. The adoption of tillage favored the mineralization process in the spring-summer period, while subsoiling favored the immobilization of carbon and nitrogen in the same period. The increase in biodiversity and microbial activity with organic fertilization is also confirmed by the results obtained from the wheat-potato rotation study, which found higher soil CO_2 emissions in this practice, which, however, may be associated with higher levels of carbon stock in the long term. Among soil tillage was the subsoiling that produced the lowest CO_2 emissions and increased soil carbon, however, the best performance in terms of agri-products produced (relative to wheat) per unit CO_2 was determined by spading and plowing and organic fertilization. In wheat cultivation, there were also benefits in terms of soil carbon balance (input/output of C into the soil) under dry weather conditions, while in potato, dry weather conditions performed well in terms of agri-food production per unit CO_2. The different climatic seasons also influenced all quality and quantity characteristics monitored on wheat. Grain yield was higher in the season with higher rainfall, while higher protein contents were found when higher water stress occurred during grain filling. Starch content showed the same trend as protein content, while lipids showed a positive relationship with grain yield. In this trial, fungicide treatments showed no significant differences, and in general, organic treatment has always demonstrated good value in terms of quality and quantity.

In general, the aim and the objectives of the thesis have been achieved. It has been shown how different agronomic practices and climatic trends can affect the system, related to production, but also to the characteristics of the soil covered. Climatic fluctuations from year to year were the factors that most influenced quantitative performance.

Despite the commitment of scientific research, sustainable management still requires complex and often not univocally definable solutions. This is why it is important to continue to evaluate the responses of the agroecosystem factors, in particular, in a rotation system such as the one studied it is important to study the sustainability of the system in the long term, to assess all the interactions of the system as a whole. Furthermore, for varietal selection, it is important to carry out studies involving a substantial number of genotypes tested in a set of cultivation environments representative of the different pedoclimatic conditions for selected varieties that can be defined as "HPLW" High-Performance and Low-Risk. When managing a cultivation system, it must always be considered that the plant-soil system is an open system and although this is widely studied, it is not yet completely known, because it is governed by very complex physical, chemical, and biological mechanisms. Input substitution is possible and desirable if the change is in line with overall goals and philosophy and if workforce, equipment, and management skills are available to implement the change. Taken together, the results show that sustainable practices, in the Mediterranean environment, can be adopted with good results in wheat and potato rotation systems and wheat cultivation. The results could specifically help reduce mineral fertilizer inputs where possible and encourage more conservative tillage. Reduced inputs will result in reduced costs for farmers as well as reduced environmental impact. In addition, these will have a positive impact on food security. The assessments conducted can help to better understand the environmental impacts that agricultural management has on the different components of the system to choose the best alternatives to enhance production, quality and economic-environmental impacts. However, since climate fluctuations have been the most influential factors in quantitative performance, future research should increasingly consider the impact of environmental variability.

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Complete list of Publications

1. Assistance Needed for Increasing Knowledge of HACCP Food Safety Principles for Organic Sector in Selected EU Countries.
   Allam M., Bazok R., Bordewick-Dell U., Czarniecka-Skubina E., Kazimierczak R., Laikoja K., Luik A., Mrkonjić Fuka M., Muleo R., Peetsmann E., Petroselli V., Roasto M., Średnicka-Tober D., Veith M., Mancinelli R., Trafialek J.
   Sustainability 2023, 15(8), 6605; https://doi.org/10.3390/su15086605

2. Durum wheat–potato crop rotation, soil tillage, and fertilization source affect soil CO_2 emission and C storage in the mediterranean environment.
   Mancinelli R., Marinari S., Atait M., Petroselli V., Chilosi G., Jasarevic M., Catalani A., Abideen Z., Mirzaei M., Allam M., Radicetti E.
   Land 2023, 12(2), 326; https://doi.org/10.3390/land12020326

3. Durum wheat production as affected by soil tillage and fertilization management in a mediterranean environment.
   Mancinelli R., Allam M., Petroselli V., Atait M., Jasarevic M., Catalani A., Marinari S., Radicetti E., Jamal A., Abideen Z., Chilosi G.
   Agriculture 2023, 13(2), 433; https://doi.org/10.3390/agriculture13020433

4. Influence of organic and mineral fertilizers on Soil Organic Carbon and crop productivity under different tillage systems: a Meta-analysis.
   Allam M., Radicetti E., Quintarelli V., Petroselli V., Marinari S., Mancinelli R.
   Agriculture 2022, 12(4), 464; https://doi.org/10.3390/agriculture12040464

5. Microbial Indices to Assess Soil Health under Different Tillage and Fertilization in Potato (Solanum tuberosum L.) Crop.
   Marinari, S., Radicetti, E., Petroselli, V., Allam, M., & Mancinelli, R.
   Agriculture 2022, 12(3), 415; https://doi.org/10.3390/agriculture12030415

6. Meta-analysis approach to assess the effects of soil tillage and fertilization source under different cropping systems.
   Allam M., Radicetti E., Petroselli V., Mancinelli R.
   Agriculture 2021, 11(9), 823; https://doi.org/10.3390/agriculture11090823

7. Weed spectrum in durum wheat under different soil tillage and fertilizer application in mediterranean environment.
Petroselli V., Radicetti E., Safahani Langeroodi A., Allam M., Mancinelli R. **Sustainability 2021**, 13(13), 7307; https://doi.org/10.3390/su13137307