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Role of multiple cropping in the conservation of soil fertility and in the reduction of greenhouse gas emissions

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Abstract

The conservation of soil fertility is a pressing challenge in modern agriculture, particularly in the context of climate change and soil degradation. Intensive farming practices have contributed to declining soil organic matter and increased vulnerability to extreme weather events. Addressing these issues requires the adoption of sustainable management practices that enhance soil health, improve nutrient cycling, and promote climate resilience. Multiple cropping systems, especially those integrating cover crops, represent a promising solution, as they improve soil structure, increase organic carbon sequestration, and reduce nutrient losses. However, the effectiveness of these strategies depends on factors such as species selection, management practices, and site-specific conditions. This Ph.D. research investigated the role of multiple cropping systems, with a focus on cover crops, in enhancing soil health, sustaining crop productivity, and mitigating environmental impacts. Two field experiments were conducted in contrasting agroecosystems, a conventional cereal-based system and a lawn-managed system, to evaluate the effects of cover crops and other soil-enhancing strategies on soil chemical, biological, and physical properties as well as crop performance.

The first experiment assessed the performance of different cover crops in a lawn-managed system, comparing various clover species and mixtures against a clover monoculture. Particular attention was paid to their effects on soil organic carbon (SOC) dynamics, carbon sequestration, biomass production, and soil water retention. Results demonstrated that species selection strongly influenced soil health outcomes. Crimson clover (Trifolium incarnatum L.) achieved the highest aboveground biomass, while tillage radish, despite lower aboveground biomass, significantly improved SOC fractions, soil water storage, and annual carbon sequestration (4.58 t CO_2 ha^{-1} y^{-1}).

The tap-root system of tillage radish (Raphanus sativus Var.Longpinnatus L.H.Bailey) enhanced soil porosity and introduced root-derived carbon inputs, delivering multifunctional benefits. In contrast, grass-based or mixed-species treatments showed variable effects, and in some cases even resulted in net carbon loss, suggesting that a single growing season may not be sufficient for measurable improvements in soil function.

Following these findings, tillage radish was further evaluated in a conventional cereal-based cropping system under contrasting tillage regimes. Tillage radish performed more effectively under conventional tillage, where increased soil disturbance favored root development, biomass production, and nutrient mobilization. Improvements were recorded in SOC, labile carbon fractions, microbial biomass, and nutrient availability, particularly in deeper soil layers. Under reduced tillage, however, plant growth was consistently limited, particularly in drier conditions, likely due to restricted root penetration and slower mineralization of organic matter. Seasonal was also important: wetter periods enhanced shoot growth and nutrient uptake, while drier periods promoted root allocation.

A broader multi-site analysis confirmed that tillage radish effectiveness is highly site-dependent. Soil texture, salinity, compaction, and climatic conditions significantly influenced biomass production and nutrient uptake. In finer-textured, low-salinity soils with favorable rainfall patterns, tillage radish showed greater biomass accumulation and nutrient translocation. Across sites, tillage radish increased labile carbon fractions (ΔPOM-C, ΔPOX-C) and microbial biomass pools (ΔMBC, ΔMBP, ΔMBN), particularly where biomass thresholds were met. However, in heavier soils and under biomass-limited conditions, soil improvements were constrained, reinforcing the need for effective residue management.

Tillage radish also demonstrated a dual role in nitrogen dynamics, enhancing nitrogen retention and microbial nitrogen accumulation while posing nitrate leaching risks under heavy rainfall or poor residue management. This highlights the need for carefully tailored management strategies to maximize benefits while minimizing environmental risks.

To complement these findings, an additional study assessed the integration of sustainable practices, including cover cropping, reduced tillage, crop rotation, and alley cropping, on soil and crop performance in Mediterranean cereal systems. When sown between crop cycles, tillage radish improved the grain and protein yields of spring barley, likely due to enhanced nutrient availability and improved soil structure. While conventional tillage initially led to higher yields, reduced tillage offered greater resilience under waterlogged conditions, suggesting long-term soil structural benefits. At the system level, crop rotations and olive-based alley cropping maintained productivity while improving nitrogen use efficiency and soil quality. In contrast, hazelnut-based alley cropping reduced herbaceous crop yields due to competition, although legume inclusion partially mitigates this effect.

Overall, this research confirms the multifunctional role of tillage radish in conservation agriculture, particularly in enhancing soil fertility, promoting carbon sequestration, and improving nutrient cycling. Species selection, pedoclimatic conditions, and tailored management practices emerged as critical factors for maximizing benefits. These findings contribute robust, field-based evidence supporting the integration of multiple cropping systems and soil-improving practices in sustainable agriculture strategies aligned with the goals of the Common Agricultural Policy (CAP 2023–2027).

Riassunto

La conservazione della fertilità del suolo rappresenta una sfida urgente nell'agricoltura moderna, specialmente nel contesto dei cambiamenti climatici e del degrado del suolo. Le pratiche agricole intensive hanno contribuito alla diminuzione della sostanza organica del suolo e a una maggiore vulnerabilità agli eventi meteorologici estremi. Affrontare queste problematiche richiede l'adozione di pratiche di gestione sostenibile che migliorino la salute del suolo, favoriscano il ciclo dei nutrienti e promuovano la resilienza climatica. I sistemi colturali, in particolare quelli che integrano colture di copertura, rappresentano una soluzione promettente, in quanto migliorano la struttura del suolo, aumentano il sequestro del carbonio organico e riducono le perdite di nutrienti. Tuttavia, l'efficacia di queste strategie dipende da fattori quali la scelta delle specie, le pratiche di gestione e le condizioni specifiche del sito. L'attività di dottorato ha indagato il ruolo dei sistemi colturali multipli, con un focus sulle colture di copertura, nel miglioramento della salute del suolo, nel mantenimento della produttività agricola e nella mitigazione degli impatti ambientali. Sono stati condotti due esperimenti in campo in agroecosistemi contrastanti: un sistema convenzionale a base di cereali e un sistema gestito a prato, per valutare gli effetti delle colture di copertura e di altre strategie di miglioramento del suolo sulle proprietà chimiche, biologiche e fisiche del suolo, nonché sulle prestazioni colturali.

Il primo esperimento ha valutato le prestazioni di diverse colture di copertura in un sistema gestito a prato, confrontando varie specie e miscele di trifoglio con una monocoltura di trifoglio. Particolare attenzione è stata rivolta agli effetti sulla dinamica del carbonio organico del suolo (SOC), al sequestro del carbonio, alla produzione di biomassa e alla ritenzione idrica del suolo. I risultati hanno dimostrato che la scelta delle specie influenza fortemente gli esiti sulla salute del suolo. Il

trifoglio incarnato (*Trifolium incarnatum* L.) ha raggiunto la maggiore biomassa epigea, mentre il rafano da dissodamento (o rafano aratore; *Raphanus sativus* Var.Longpinnatus L.H.Bailey), nonostante una biomassa epigea inferiore, ha migliorato significativamente le frazioni di SOC, la capacità di ritenzione idrica e il sequestro annuo di carbonio (4,58 t CO_2 ha^{-1} $anno^{-1}$). Il suo apparato radicale fittonante ha aumentato la porosità del suolo e introdotto apporti di carbonio derivati dalle radici, offrendo benefici multifunzionali. Al contrario, i trattamenti a base di graminacee o a specie miste hanno mostrato effetti variabili e, in alcuni casi, persino una perdita netta di carbonio, suggerendo che una singola stagione colturale possa non essere sufficiente per ottenere miglioramenti misurabili nella funzionalità del suolo.

Alla luce di questi risultati, il rafano aratore è stato ulteriormente valutato in un sistema colturale convenzionale a base di cereali, sotto regimi di lavorazione del suolo contrastanti. Il rafano ha ottenuto migliori risultati con la lavorazione convenzionale, dove il maggiore disturbo del suolo ha favorito lo sviluppo radicale, la produzione di biomassa e la mobilizzazione dei nutrienti. Sono stati osservati miglioramenti nel SOC, nelle frazioni di carbonio labile, nella biomassa microbica e nella disponibilità di nutrienti, specialmente negli strati più profondi del suolo. Tuttavia, con la lavorazione ridotta, la crescita delle piante è risultata costantemente limitata, soprattutto in condizioni più secche, probabilmente a causa di una penetrazione radicale ridotta e di una più lenta mineralizzazione della sostanza organica. Anche la stagionalità ha giocato un ruolo importante: i periodi più umidi hanno favorito la crescita epigea e l’assorbimento di nutrienti, mentre quelli più secchi hanno promosso lo sviluppo radicale.

Un’analisi più ampia su più siti ha confermato che l’efficacia del rafano aratore dipende fortemente dal sito. Tessitura del suolo, salinità, compattazione e condizioni climatiche hanno influenzato significativamente la produzione di biomassa e l'assorbimento dei nutrienti. Nei suoli a tessitura fine, a bassa salinità e con piovosità favorevole, il rafano ha mostrato una maggiore accumulazione di biomassa e traslocazione di nutrienti. In tutti i siti, ha aumentato le frazioni di carbonio labile (ΔPOM-C, ΔPOX-C) e i pool microbici (ΔMBC, ΔMBP, ΔMBN), in particolare quando si raggiungevano soglie critiche di biomassa. Tuttavia, in suoli più pesanti o in condizioni di limitata produzione di biomassa, i miglioramenti del suolo risultavano ridotti, evidenziando l'importanza di una gestione efficace dei residui.

Il rafano ha anche mostrato un duplice ruolo nella dinamica dell'azoto, favorendo la ritenzione di azoto e l'accumulo microbico, ma comportando anche rischi di lisciviazione di nitrati in caso di piogge intense o di cattiva gestione dei residui. Ciò evidenzia la necessità di strategie di gestione attentamente calibrate per massimizzare i benefici riducendo al minimo i rischi ambientali.

A complemento di questi risultati, uno studio aggiuntivo ha valutato l'integrazione di pratiche sostenibili – incluse colture di copertura, lavorazione ridotta, rotazioni colturali e sistemi agroforestali a viali (alley cropping) – sulle prestazioni del suolo e delle colture nei sistemi cerealicoli mediterranei. Quando seminato tra i cicli colturali, il rafano ha migliorato la resa in granella e il contenuto proteico dell'orzo primaverile (*Hordeum vulgare* L.), probabilmente grazie a una maggiore disponibilità di nutrienti e a una migliore struttura del suolo. Sebbene la lavorazione convenzionale abbia inizialmente portato a rese maggiori, la minima lavorazione ha offerto maggiore resilienza in condizioni di ristagno idrico, suggerendo benefici strutturali nel lungo termine. A livello di sistema colturale, le rotazioni colturali e i sistemi agroforestali con olivo hanno mantenuto la produttività migliorando al contempo l'efficienza d'uso dell'azoto e la qualità del suolo. Al contrario, i sistemi a viali con nocciolo hanno ridotto le rese delle colture erbacee a causa della competizione, anche se l'inclusione di leguminose ha parzialmente mitigato questo effetto.

In sintesi, questa ricerca conferma il ruolo multifunzionale del rafano aratore nell'agricoltura conservativa, in particolare nel miglioramento della fertilità del suolo, nella promozione del sequestro del carbonio e nell'ottimizzazione del ciclo dei nutrienti. La scelta delle specie, le condizioni pedoclimatiche e le pratiche gestionali personalizzate sono emersi come fattori critici per massimizzare i benefici. Questi risultati offrono solide evidenze sul campo a supporto dell'integrazione dei sistemi colturali multipli e delle pratiche migliorative del suolo nelle strategie agricole sostenibili, in linea con gli obiettivi della Politica Agricola Comune (PAC 2023–2027).

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1. General Introduction

1.1. Historical context

1.1.1. Historical Pressures and Early Innovations in Agriculture

Global agriculture is currently facing a number of pressures that threaten its long-term sustainability (Walsch et al. 2020). Historically, the primary focus of agriculture was ensuring food security and maximizing agricultural productivity, which led in the widespread adoption of new practices and technologies across various regions. One of the earliest agricultural innovations was the practice of cultivating and harvesting multiple crops within a single year, a technique known as multiple cropping (Waha et al. 2020).

The primary goal of agriculture, dating back to the times of Hesiod (8^{th}–7^{th} century BC) and Cato (3^{rd}–2^{nd} century BC) in old Greece and Rome, respectively, has always been to restore the "old strength" of the soil, its natural fertility lost through continuous cultivation (Landi 1997). This goal has led to agricultural practices aimed at maximizing agricultural productivity while depleting less environmental resources. Early farmers observed that cultivating the same crop repeatedly on the same soil, year after year, led to declining crop yields (Giardini 2004). To address this, different civilizations found innovative solutions.

For instance, the Hebrews, living in the Levant region (Middle East region) during the late 2^{nd} to early 1^{st} millennium BC, left their land fallow every seventh year, a practice recorded in the Torah, as a method to rejuvenate soil fertility (Borowski 1979; Turkowski 1969). Their agricultural system was centered around the cultivation of what came to be known as the Seven Species—wheat, barley, grapevines, figs, pomegranates, olives, and dates—which together provided more than 90% of locally produced food (Solowey 2016). These crops were not only vital for sustenance but also well-adapted to the arid environment. In northeastern Africa, the ancient Egyptians (from 3rd to 1st millenium BC) were among the earliest civilizations to study how specific crops influenced subsequent harvests on the same plot. However, their agricultural production was limited water scarcity and sandy, porous soil, conditions that restricted them to a single harvest per year (Dalrimple 1971). Despite these challenges, innovations in irrigation and other techniques enabled the cultivation of more than one crop each year. The Greeks (8th –4th century BC) predominantly practiced a crop-and-fallow method, where the land was cultivated for one year with cereals or pulses and left fallow the next. From the 1st century BC, the Romans gradually developed a more advanced system, recognizing the advantages of a three-course rotation (Fussel 1967). This allowed for two years of cropping with cereals or legumes, followed by a fallow year or a catch crop of legumes after the grain harvest, used as feed for the animals (Ralls 2013). Common crops included wheat, barley, and rye in the autumn, while peas, beans, and lentils were planted in spring. In India, the earliest recorded reference of multiple cropping, specifically double cropping (the cultivation of two crops on the same field within a year), dates back to 1000 BC (ICAR 1964). Over time, this approach evolved into deeply rooted traditional systems such as "Barahnaja", which literally means "twelve grains" (Gururani et al. 2021). Practiced especially in the Himalayan regions, Barahnaja involves the simultaneous cultivation of a diverse crops, including millet, amaranth, beans, and soybean among others (Sati and Kumar 2004). This system not only enhances food and nutritional security but also promotes soil health, agro-biodiversity, and climate resilience in vulnerable ecosystems (Gururani et al. 2021).

1.1.2. Medieval and Early Modern Advances in Cropping systems

During the Middle Ages, European villages commonly divided their agricultural land into three large sectors, with one sector left fallow or fallow pasture each year, while the others were cultivated with grain crops (Homans 1936). This practice, known as the three-field system, marked a decisive advance over the earlier two-field method, in which half of the land lay fallow each season. In the new system, only one-third was left uncultivated, while the remaining land was split between an autumn crop—typically wheat, rye, or barley—and a spring crop—usually oats, barley, or nitrogen-fixing legumes such as peas and beans (Encyclopaedia Britannica). It was not until the 16th century AC that systematic crop rotation was described in the works of Gallo (1572) and Tarello (1567). However, widespread adoption of crop rotation took time. A major turning point came in the first half of the 18th century with the introduction of the so-called Norfolk rotation in England (Bax and Fairfield 1978). This four-year rotation alternated cereals, brassicas, and legumes in a mixed arable/pasture rotation, which significantly improved yields and was rapidly embraced by farmers (Bax and Fairfield 1978; Riches 1967). The system was popularized in England during the 18th century by the British agriculturist Charles Townshend (1674–1738), who played a crucial role in promoting it as a cornerstone of agricultural modernization. The rotation sequence—typically wheat, turnips, barley, and clover—included both a fodder crop and a grazing crop, allowing for year-round livestock breeding. This innovation made the four-field system one of the central developments of the British Agricultural Revolution. In addition, during the third cropping season, clover was sown in the winter wheat field between the tillering and stem elongation stages, improving soil nitrogen levels and providing additional forage. In Italy, Ridolfi (1968) promoted a continuous crop rotation system based on the Norfolk system, adapting it to local conditions in Northern Italy by incorporating legumes such as alfalfa, sainfoin, and sulla (Landi 1997). These adaptations contributed to overall improvements in soil fertility. This is why Ridolfi (1968) referred to the accumulated fertility as "calorie", a precious reserve that should not be wasted due to poor agricultural management or excessive short-term greed. Many ancient cropping systems also had limitations that would challenge their application in the modern world. In several cases, their environmental impacts—such as soil degradation from prolonged fallow periods or overreliance on monoculture staples—were not fully understood at the time. Moreover, some of these systems depended on socio-cultural that are no longer present or replicable today. For example, the fallow-based rotations of the Greeks or Romans, while effective in conserving soil fertility, may not meet the productivity demands of current global food systems. These historical examples, therefore, offer valuable insights but also underscore the need to critically assess their relevance in the context of today's complex environmental and socioeconomic challenges.

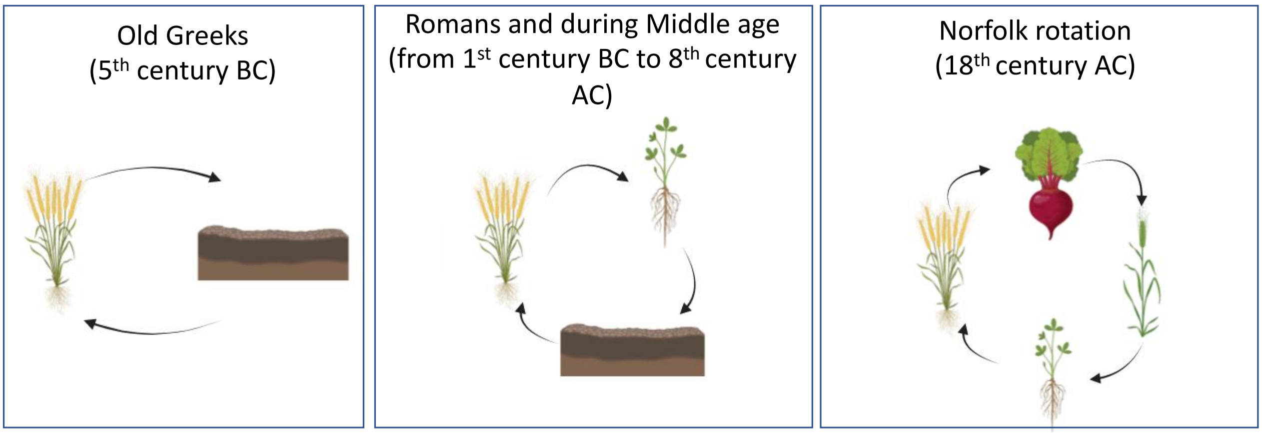

Figure 1 Schematization of the major historical changes in cropping systems

1.1.3. Modern Advances in Cropping systems

More recently, crop productivity boosted significantly by implementing various strategies, including the expansion of arable land area (John and Babu 2021). Since 1850, global land use has been characterized by the decline of natural areas (non-agricultural and non-urban land) alongside the expansion of agricultural areas, an ongoing process known as extensification (Taylor and Rising 2021). The expansion of agricultural land was a direct consequence of the growing population that rose from 1 billion in 1800 to 2.2 billion by 1938 (Federico and Tena-Junguito 2022).

Although early advances in soil chemistry and agronomy were made by scientists such as von Liebig and Boussingault, organic fertilizers (e.g., animal manure, crop residues, green manures) remained dominant until the early 1900s (Borlaug 2002). The introduction of synthetic fertilizers only became widespread after Fritz Haber and Carl Bosch developed the process for synthesizing ammonia. However, during the two World Wars, the use of fertilizers was interrupted as ammonia was diverted to nitrate production for explosives (Borlaug 2002).

The Green Revolution, that started back in the late 1940s, was an important breakpoint in global agriculture. For the first time, the global area under cultivation reached a plateau, and in some regions, such as North America, Eurasia, and China, it even declined (Ramankutty et al. 2018). The development of high-yielding varieties, especially wheat and rice, was decisive in meeting the food demands of an increasing worldwide population. As Ehrlich (1968) famously observed, before the Green Revolution, the ‘battle to feed the world’ had been lost. Modern cropping systems began to emerge during that period, especially throught technology transfer initiatives like in China and India which led to a doubling of cereal production in less than a decade (Hazell 2009; Borlaug 2002). Since 1961, these gains in crop yield have been primarily driven by intensification – improving the yield of exhisting cultivated land – rather than further expansion. Globally, cropland expanded by 27%, from 960 to 1208 million hectares, while total crop yield rose by 135%, from 1.84 to 3.96 tons per hectare (Burney et al. 2010).

After the Green Revolution, the co-evolution of heavy machinery, specialized input-intensive farming systems, and the "feed the world" philosophy, defined modern industrial agriculture in the Global North (Evens-Ditzler 2022). One of the most significant consequences has been the dependence on synthetic fertilizers. While these fertilizers helped boost crop yields, they also created a cycle where soil quality is heavily dependent on chemical inputs (Borlaug 2002). The shift toward industrial agriculture and its dependence on chemical inputs may have been successful in boosting food production in the short term, but it also created a cycle that often undermines soil health and environmental sustainability (Crippa et al. 2021). Consequently, the agricultural sector is now facing the challenge of transitioning to more sustainable practices that can break this cycle and ensure long-term food security without further harm to the environment.

1.2. The Sustainable Intensification need of the cropping systems

Although the Green Revolution achieved significant successes by dramatically increasing food production and averting famines in many parts of the world, it left unresolved the profound challenge of ensuring food security for hundreds of millions of people living in extreme poverty (Borlaug 2002). This challenge remains urgent today, as farmers worldwide face mounting pressure to ensure an adequate food supply for a growing global population, which is projected to approach 9.8 billion by 2050 (United Nations, 2019). Meeting this demand requires an unprecedented increase in food production, with estimates suggesting that in 2050, food output must increase by over 50% compared to 2010 levels (Searchinger et al. 2018).

In terms of annual crop calorie production, recent projections estimate a 56% food gap by 2050, compared to 2010. This figure represents the difference between the amount of food required provide adequate nutrition for the global population in 2050 and the quantity produced in 2010

(Searchinger et al. 2018). Achieving this goal without compromising environmental integrity is an ambitious challenge, highlighting the need for transformative agricultural strategies that balance productivity with sustainability (Pretty and Bharucha 2014).

Agriculture, which currently employs approximately 1 billion people (FAO 2024), must remain a driving force for global development, while simultaneously reducing its environmental footprint. However, the agrifood sector remains a major contributor to greenhouse gas (GHG) emissions, accounting for nearly one-third of global emissions (Crippa et al. 2021), with previous estimates placing this figure at about one-quarter (UNEP 2012). As a result, agriculture is increasingly threatening the agroecosystems that support the production of food, feed and fiber.

Key environmental concerns include land degradation, water scarcity and loss of biodiversity, all of which weaken the resilience of the cropping systems (Walsch et al. 2020). Some agricultural practices have been identified as significant contributors to ecosystem destabilization. Willett et al. (2019), for instance, highlight that current food production system poses a significant threat to both climate stability and ecosystem resilience. Without major changes, agricultural expansion is likely to continue affecting these essential ecological functions, with potential severe consequences for global ecosystems (Sala et al. 2000).

To prevent further damage, agriculture should aim to halt net expansion of agricultural land, thereby protecting the natural ecosystems that provide the critical services underpinning food production (Searchinger et al. 2018). Over the past five decades, agriculture has become more land-efficient thanks to significant yield improvements (Viana et al. 2022). Nevertheless, projections indicate that global cropland could expand by as much as 593 million hectares by 2050, even if yield gains continue at historical rates (Figure 2). In more pessimistic scenarios, where yield growth slows, agricultural land expansion could be even greater, threatening the world's remaining forests and savannas.

These projections reinforce the urgent need for both yield improvement and sustainable management practices to mitigate agricultural land expansion and prevent further environmental degradation. Some studies suggest that pursuing a trajectory of technological innovation and yield intensification in underperforming regions could help meet global food demand at a significantly lower environmental costs (Tilman et al. 2011). Strategic intensification, enhancing yields in low-productivity nations through technology transfer, soil fertility improvements, and efficient nutrient management, could reduce the need for land clearing, limit GHG emissions, and decrease biodiversity loss (Tilman et al. 2011).

In this context, multiple cropping systems represent a tangible solution to increase agricultural productivity without exacerbating environmental impacts, offering a viable pathway toward food security, resource efficiency, and ecosystem conservation.

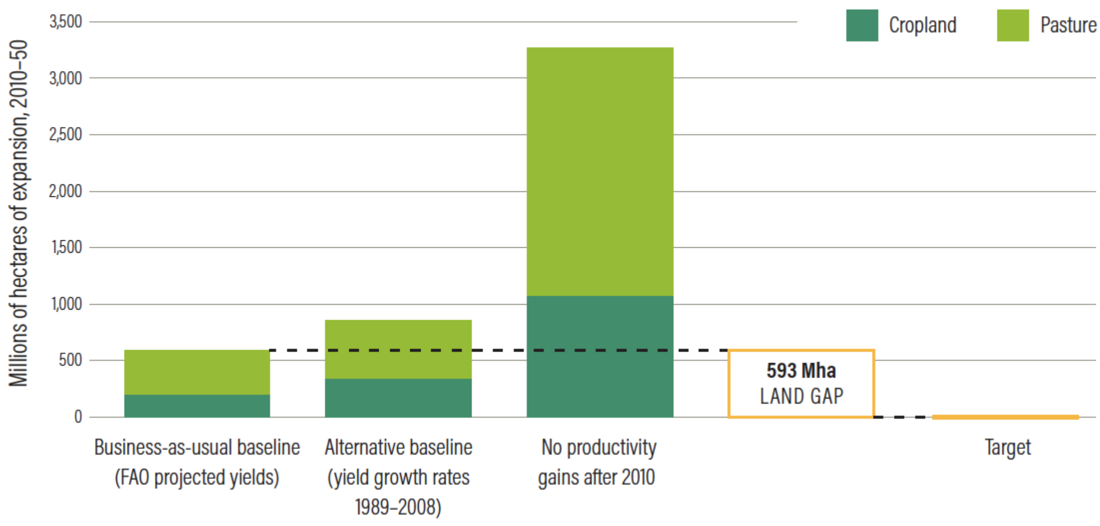

Figure 2 Different scenarios of agricultural land expansion. (From Searchinger et al. 2018)

1.3. Multiple cropping systems

The prevailing model of the agricultural systems is rooted in high-input practices, such as frequent tillage, synthetic agrochemicals, irrigation, and simplified cropping schemes (Rockström et al. 2017). One example of such simplification is the cultivation of the same crop or cultivar on the same land year after years, a practice known as monocropping (Encyclopaedia Britannica). The simplification of the cropping systems was a direct consequence of the specialization of the farmers in one or two crops, often facilitated by the adoption of advanced machinery (Paul et al. 2024). This led to the widespread adoption of monocropping, achieved through the mechanization of tilling, fertilizing, planting, pesticide applications, and harvesting. Traditional practices, such as diversifying cropping system to meet the varying nutrient requirements of different crops, were largely abandoned, due to the increasing availability and attractiveness of cheap synthetic fertilizers and pesticides (Horwith 1985).

Today, rising energy costs and growing concerns about the environmental impacts of these inputs have renewed interest in traditiona, diversified practices (Shennan 2008). As a result, the studies on ecosystem services provided by the cropping system diversification and intensification through multiple cropping has expanded considerably (Blanco-Canqui et al. 2015; Gaba et al. 2015). Multiple cropping systems involve cultivating two or more plant species or varieties in a coordinated manner, accounting for both spatial and timing dimensions (Gaba et al. 2015). This can include growing different crops together in the same field during a single growing season, with an emphasis on spatial arrangement (Ghaley et al. 2005). Within this framework, three common approaches to intercropping can be identified (Toker et al. 2024):

1. mixed intercropping, where two or more crops are grown together in the same area without any distinct row arrangement. For example, planting beans alongside maize (corn) in the same area. The beans can fix nitrogen, which benefits the maize, and the maize provides support for the beans to climb (Fischer et al. 2020).;
2. row intercropping, which involves growing different crops in alternating rows or strips to get benefits of multiple crops while facilitating management and harvesting. An example of such system is the practice of growing practice of growing wheat and clover in alternating rows (Thorsted et al. 2006);
3. relay intercropping, where a second crop is sown into a standing crop before the first one is harvested, allowing for overlapping growth periods and more efficient use of resources. For example, sowing a fast-growing legume like soybeans into a field of maize about halfway through the maize's growing season (Ping et al. 2018). By the time the maize is harvested, the soybeans are well-established and ready to continue growing

However, modern cropping systems are generally not designed to support these intercropping approaches effectively, either agronomically or technologically (Brooker et al. 2015). As a result, some studies have reported reduced crop performance following the adoption of intercropping, often due to mismanagement or suboptimal conditions (Li et al. 2023; Blanco-Canqui 2024).

In the Mediterranean region, for instance, water scarcity during key periods significantly limits the feasibility of these practices, as competition for limited water resources between different crops can negatively impact their performances (Blanc et al 2024; Bedoussac and Justes, 2011). Moreover, intercropping adoption by European farmers remains limited, largely due to insufficient knowledge and technical support among both farmers and advisors (Ha et al. 2023). Other alternatives for implementing multiple cropping system, such as the use of cover crops to enhance soil health and further diversify crop production, are discussed in the following sections.

1.4. Cover cropping for multiple cropping

1.4.1. Classification of Cover Crops and Their Framework Within the Common Agricultural Policy

A practical approach to achieving cropping system intensification is the adoption of well-established practices, such as cultivating crops during fallow periods between main crop cycles—commonly referred to as 'interim crops' or 'cover crops' (CCs). These practices optimize land use, enhance soil fertility (Pisarčik et al. 2024), and promote biodiversity while increasing cropping frequency, potentially transitioning from a conventional monocropping system to a double-cropping system. In some cropping system of the Mediterranean basin, up to nine months can pass between the wheat harvest and the planting of the next cash crop, a period during which the soil is often left uncultivated (Leoni et al. 2022). This long fallow period offers an opportunity to intensify traditional cropping systems while enhancing soil properties. Cultivation during this period, often referred to as cover cropping, involves cultivating non-harvestable crops grown for various purposes such as nutrient uptake, soil protection, and erosion control (Young et al. 2021; Fageria et al. 2005).

Crops that are not intended for being harvested fall into the category of CCs and are further classified based on their primary functions (Ramírez-García et al. 2015). A specific subset of CCs known as catch crops is cultivated to absorb available nitrogen (N) from the soil, thereby reducing nitrogen leaching losses (Thorup-Kristensen et al. 2003). Another subset of cover crops, green manure crops, is cultivated to improve soil nutrient content and organic matter levels, thereby improving the soil fertility for subsequent main crops (Thorup-Kristensen et al. 2003).

Table 1 presents a classification of cover crops by specific purposes and their key agronomic requirements. The practice of sowing CCs is an integral part of various sustainable agricultural approaches, including conservation and regenerative agriculture (Corsi and Muminjanov 2019; Giller et al. 2021). However, further scientific evidence is needed to support widespread adoption by farmers. Combining CCs with reduced tillage or no-till practices plays an importat role in maintaining continuous soil cover, which contributes to improving soil properties — a topic explored further in the following paragraph. In addition to their agronomic value, CCs have also been promoted through policy frameworks. Under the 2023-2027 Common Agricultural Policy (CAP) framework, CCs use falls under enhanced conditionality, a set of environmental and agronomic requirements that farmers must meet to access subsidies (EU Commission, 2023). Specifically, CCs contribute to compliance with GAEC 6 (Good Agricultural and Environmental Condition 6), which mandates maintaining a minimum soil cover during sensitive periods to protect soil fertility and prevent erosion. In Italy, GAEC 6 applies to arable land, requiring at least 60 consecutive days of soil cover between September 15th and May 15th (EU Commission, 2023).

Table 1 Classification of cover crops by functions and agronomic requirements (Adapted from Ramírez-García et al. 2015)

Use	Function	Requirements
Cover crop	- Erosion control - Increase soil organic matter	- Fast and high ground cover and slow decay residues
Catch crop	- Nutrient/pollutant uptake	- High biomass accumulation and N uptake
Green manure	- Nutrient supply - Increase soil organic matter	- High N uptake and/or supply and easily decomposable residues
Fodder	- Forage production	- High biomass accumulation and dietary fiber content

1.4.2. The benefits of cover cropping

As this thesis examines the various functions outlined in Table 1, the term 'cover crops' will be used to encompass practices aimed at nutrient cycling, enhancing soil organic carbon (SOC), and improving soil health in Mediterranean agricultural systems. In this region, soil degradation, nutrient depletion, and declining organic matter content are significant challenges, making CCs a valuable tool for sustainable agriculture (Ferreira et al. 2022).

Additionally, an important benefit not listed in Table 1 is that CCs contribute to increased diversification of cropping systems, which are often dominated by monocropping (Omer et al. 2024). By introducing varied root structures and biochemical interactions, CCs can help disrupt soilborne pathogen cycles, ultimately enhancing the performance of subsequent main crops (Qi et al. 2020).

Cover crops can also be categorized based on their species composition, namely as single-species or multi-species mixtures (Koudahe et al. 2022). While many authors have emphasized the benefits of diversified cropping systems, prompting numerous studies on the benefits of multi-species mixtures (Trenbath 1974; Tilman et al. 2014; Miyazawa et al. 2014), the effects of specific single-species CCs on soil properties and ecosystem services under different pedoclimatic conditions remain underexplored. This gap in research is particularly evident in the European context, as most

CC trials have been conducted in North and South America, regions with broader CC adoption among farmers (Zhou et al. 2022). This lack of region-specific data highlights the urgent need for localized studies across European agroecosystems, especially in light of the evolving Common Agricultural Policy (CAP), which increasingly promotes eco-schemes and sustainable practices. Without robust local evidence, it remains difficult to design effective policy incentives or provide clear agronomic recommendations to European farmers.

Accordingly, Gutknecht et al. (2022) conducted farm and soil surveys in Minnesota to examine relationships between soil health and CC sowing. Their findings highlighted that soil health responses to CC implementation vary due to multiple factors, including tillage practices, crop rotation, planting timing, and site-specific edaphic characteristics such as soil texture and topography. Single species CCs are usually grouped into leguminous broadleaves, non-leguminous broadleaves, and grasses (Koudahe et al. 2022). The choice of CC depends on the intended agronomic or ecological objective, as previously outlined in Table 1, but also on the crop types that are adapted and available within specific local context.

1.4.3. Legumes for cover cropping

In the Mediterranean context, CCs are primarily used to increase nitrogen inputs throught legume-based nitrogen fixation and enhance overall soil health (Curtright et al. 2025). As such, legume-based CCs have attracted significant attention for their ability to reduce nitrogen fertilizer needs for the main crops by fixing atmospheric nitrogen (Hansen et al. 2021; Blanco Canqui et al. 2015; Dabney et al. 2001). Additionally, legume CCs can minimize nitrogen leaching and optimize nitrogen retention in the root zone (Nyfeler et al. 2024; Lacey and Armstrong 2015).

Kirkegaard et al. (2008) found that integrating legumes into a cropping system can boost the yield of subsequent cereal crops by an average of 24% compared to monocropping. Common leguminous cover crops are species include clovers, cowpea, faba bean, alfa-alfa, peas, soybean, sunn hemp, and vetch (Koudahe et al. 2022).Several studies also link the use of legume CCs to increased soil organic carbon (SOC) (Blanco-Canqui et al. 2022; Elhaddad et al. 2024; De Bernardi et al. 2025), though results vary (Di Rauso Simeone et al. 2020).

1.4.4. Cover crops effect on soil organic carbon dynamics

The role of CCs in promoting SOC sequestration remains debated. Blanco Canqui et al. (2015) identified insufficient soil sampling as a key reason why short-term variations in SOC often go undetected. This highlights the need for well-designed experimental designs with intensive and spatially distributed soil sampling to capture subtle changes in SOC dynamics.

Since total SOC is slow to respond, labile SOC fractions offer a more sensitive indicator of short-term soil health changes. In a meta-analysis, Blanco Canqui (2023) found that in 58% of studies, reportedincrease in labile soil organic carbon following CC adoption. Particular attention has been given to particulate organic matter (POM) and its associated carbon (C) fraction (POM-C), which originate from organic amendments and crop residues (Derrien et al. 2023). The POM-C and serves as a key indicator of short-term soil health improvements. Since POM-C is highly responsive to organic inputs, the incorporation of CCs can lead to a rapid and detectable increase in this fraction, thereby enhancing soil carbon dynamics and nutrient availability. Another important labile carbon fraction is permanganate-oxidizable carbon (POX-C), which reflects microbially available carbon pool (Inagaki et al. 2021) and is sensitive to management-induced changes, such as tillage and cover cropping (Lucas and Weil 2021). To gain a comprehensive understanding of the carbon dynamics in soil, it is also essential to consider microbial biomass carbon (MBC), which represents the carbon contained within soil microbial communities (Joergensen et al. 2024). The MBC is itself a labile C pool in soil, comprising the carbon of bacteria, actinomycetes, fungi, algae, protozoa, and other soil microfauna (Das et al. 2023). Importantly, the C derived from CCs can serve as a source of energy for the microorganisms in soil, which in turn contributes to microbial biomass formation and turnover (Linsler et al. 2016). The CCs' residue quality and quantity influence microbial abundance and community structure, affecting overall soil function (Torabian et al. 2025).

1.4.5. Non-Leguminous cover crops

Non-leguminous CCs have demonstrated positive effects not only on the aforementioned carbon fractions together but also on a range of soil properties beyond C dynamics. Several studies have shown that these species help reduce nitrate leaching across the soil profile, thereby minimizing nitrogen losses and improving nutrient retention (Dozier et al. 2017; Cooper et al. 2017). Among non-leguminous CCs, species from the Brassicaceae family have been extensively studied due to their multiple agronomic benefits (Gieske et al. 2016; Alcántara et al. 2009; Haramoto and Gallandt 2004;). In particular, daikon radish (*Raphanus sativus* L., var. longipinnatus), commonly referred to as tillage radish, has gained attention, especially in United States of America (USA), for its capacity to improve various soil properties. The specific benefits provided by tillage radish will be further analyzed in the following section.

1.5. Tillage radish as a versatile cover crop for improving soil health and achieving multiple cropping

1.5.1. Tillage radish management

The Brassicaceae family includes a diverse range of species that can be cultivated as CCs. The most commonly used brassica species include *Brassica rapa* L. (turnip, rapeseed, canola), *B. napus* L. (rapeseed, canola), *Raphanus sativus* L. (forage, oilseed, or daikon radish), and *Sinapis alba* L. (white or yellow mustard) (Gieske et al. 2016). Among these, tillage radish (*Raphanus sativus* var. longipinnatus) has received increasing attention due to its multifunctional benefits.

The management of tillage radish typically involves seedbed preparation, sowing, and, in some cases, termination through mechanical or chemical means. However, in conservation agriculture systems, soil preparation may be avoided, as these crops can be directly seeded into previous crop residues. Consequently, tillage radish has been tested in various conservation cropping systems for their potential to enhance water infiltration and reduce soil compaction (Sartori et al. 2021). Furthermore, some tillage radish cultivars are frost-sensitive that naturally die when exposed to freezing temperature. This characteristic eliminates the need for mechanical or chemical termination, thereby reducing management costs and labor requirements (SARE Outreach, 2007). When cultivated in rows, radish CCs can also facilitate the establishment of subsequent cash crops, as residual rows provide natural seedbeds (Chen and Weil 2011).

Due to their rapid growth and high biomass accumulation, even within an 11-week period (Gruver et al. 2014), tillage radish is considered an ideal cover crop for double-cropping systems in cereal-based rotations, which typically involve only one crop per year (Leoni et al. 2022). It is particularly well-suited for systems where land is left fallow between the cereal harvest in July and spring crop sowing in March, thus maximizing land use efficiency.

Tillage radish has demonstrated multiple benefits for soil properties, both through the release of glucosinolates and through improvements in physical and chemical soil characteristics (Yagioka et al. 2014; Blanco-Canqui and Ruis 2020).

Glucosinolates are bioactive compounds released during decomposing, and they have been shown to suppress various soilborne pathogens, contributing to natural soil disease suppression (Sassenrath et al. 2019). The production of glucosinolates by Brassicaceae crops and their subsequent release into the soil upon residue decomposition has been extensively documented (Bhandari et al., 2015; Antonious et al., 2009). Harbach and Tylka (2022) observed a significant reduction in plant-parasitic nematode populations following the cultivation of daikon radish as a CC, suggesting its potential for biological control. However, contrasting findings have emerged. For instance, Gruver et al. (2010) found no significant allelopathic effects on free-living nematode communities and no measurable reduction on plant-parasitic nematodes. Despite growing evidence supporting the benefits of daikon radish, a critical research gap remains regarding its repeated use within the same system, particularly in Mediterranean agroecosystem conditions.

1.5.2. Enhancing Soil Physical Properties Using Tillage Radish

Tillage radish is widely recognized for its ability to physically protect soil from erosion, shielding the surface from rain and wind (Haruna et al 2020). Upon termination, its deep taproot and leave vertical channels known as bio-pores (Han et al 2015), which improve soil aeration and facilitate root penetration for subsequent crops through a mechanism called bio-drilling (Raut and Dick, 2020; Kautz 2015). These bio-pores represent a viable alternative to conventional tillage, especially in conservation agriculture systems, where physical degradation of the soil can occur during the early transition years (Zhu et al. 2024; Gomez et al. 2001). The extensive root systems of radish can reach depths of up to 2 m (Dean and Weil 2009), playing a key role in alleviating compaction, improving aeration and water infiltration, and enhancing root penetration (Koudahe et al. 2022).

The deep taproot system of radish allows it to penetrate compacted soil layers, making it particularly effective in finer-textured soils. This species has demonstrated a notable ability to alleviate compaction, producing the greatest mean root volume within compacted zones compared to other cover crops (Burr-Hersey et al. 2017). In contrast, black oat develops a finer fibrous root system with a larger surface area, enhancing root-to-soil contact. This trait may favor nutrient and water uptake but is less effective at creating deep biopores. Tillage radish, by forming biopores, can facilitate root growth of subsequent crops by creating physical pathways in compacted soils (Burr-Hersey et al. 2017). Despite these advantages, recent studies have reported mixed results. For instance, Sartori et al. (2022) found that tillage radish did not consistently improve compaction during the transition to conservation agriculture. Moreover, in some cases, tillage radish residues were associated with reduced pore volume near the decomposed taproot zone, potentially increasing localized compaction (Vending 2019). These contrasting results highlight the need for further research across different tillage systems and pedoclimatic contexts to optimize the use of tillage radish in sustainable soil management. Beyond its physical impact, CC taproots exude organic compounds that contribute to soil particle aggregation and structural stability, thereby influencing soil structure and improving its resilience to erosion and degradation (Kirkegaard et al 2008). Upon termination, the decomposition of radish residues and the release organic compounds can stimulate microbial activity, support nutrient cycling, and improve soil aggregation, offering lasting benefits to soil health and subsequent crop productivity.

1.5.3. Enhancing Soil Chemical Properties Using Tillage Radish

Tillage radish residues decompose rapidly, facilitating the timely release of nutrients into the soil (Koudahe et al. 2022). However, the overall benefits of CCs are closely linked to their biomass production, which directly influences their impact on soil properties (Wagg et al. 2021). In this regard, Blanco-Canqui (2023) highlighted that the lack of biomass quantification in many cover crop studies represents a major limitation in assessing their contribution to soil organic matter accumulation. Notably, the distinct effects of aboveground and belowground residues on soil organic matter dynamics must be considered. Kong and Six (2010) demonstrated that root-derived carbon plays a more significant role in long-term soil fertility compared to carbon from aboveground biomass, thereby emphasizing the importance of belowground biomass in sustaining soil health. Consequently, the decomposition of radish taproots can significantly contribute to soil organic carbon accumulation and soil fertility improvement. Studies on forage radish CCs have shown their ability to increase soil organic carbon and its labile fractions, thereby improving soil physicochemical properties and nutrient availability (Wang et al. 2024). However, despite this potential, few studies have specifically quantified labile organic carbon fractions in daikon radish systems. Addressing this gap is essential to better understand its role in carbon sequestration and soil fertility, particularly under different management practices.

Crop yields are also frequently limited by the availability of key nutrients such as phosphorus (P), particularly in soils with high P fixation capacity or low natural P levels (Tóth et al. 2014). Phosphorus is a vital but often limiting nutrient in many agroecosystems (Mancini et al. 2024), and its inefficient use can result in excessive fertilizer inputs and environmental concerns. The use of CCs may mobilize P from otherwise 'unavailable' pools, although standard soil P tests may not always capture these changes (Hallama et al. 2019). The effectiveness of CCs in improving P availability depends on factors, including species selection, tillage practices, and fertilization strategies. In this context, White and Weil (2011) demonstrated that after two years of forage radish cultivation, phosphorus availability was significantly higher in the soil surrounding radish biopores compared to untreated areas. Their study, conducted in soils already high P availability, suggests that the positive effect of forage radish on P mobilization could be even greater in P-poor soils. These findings underscore the potential role of tillage radish in enhancing localized P availability through root decay and residue decomposition, ultimately benefiting nutrition and performance of subsequent crops.

In light of its multifunctional benefits—ranging from improved soil physical and chemical properties to the low management costs—the tillage radish emerges as one of the most promising options for promoting sustainable and intensified cropping systems in Mediterranean environments. Its integration into multiple cropping systems can support both environmental goals and agronomic efficiency, making it a valuable tool in the transition toward climate-resilient agriculture.

2. Objectives

This PhD thesis aims to evaluate the role of multiple cropping systems in improving soil fertility, with a particular focus on diversified cropping practices and reduced tillage strategies. As previously observed, multiple cropping systems, including cover crops (CCs) and alternative agronomic management strategies, are increasingly recognized as effective tools for enhancing soil health. The first and second objectives of the thesis focus on the effect of double-cropping through the use of cover crops on chemical, biological and physical soil properties under two different management systems. The third objective investigates the use of tillage radish as a CC within a double-cropping system to enhance soil fertility under two reduced tillage conditions.

The thesis is structured around four core research objectives:

1. Evaluate the impact of different cover crops and crop mixtures on soil fertility and carbon sequestration, by assessing their ability to improve soil physical, chemical, and biological properties. This includes investigating the potential of various clover species, tillage radish, and specific crop mixtures on key soil health indicators.
2. Assess the role of tillage radish as a cover crop in improving phosphorus availability and carbon storage under different tillage regimes. The study will analyze how tillage radish contributes to short-term C sequestration, improves soil structure, and enhances the availability of essential nutrients like phosphorus (P), as compared to conventional bare-soil management.
3. Investigate the potential of tillage radish to replace fallow periods across different soil types and climatic conditions, evaluating its ability to establish successfully in diverse

environments and its effects on key soil health indicators related to carbon (C), nitrogen (N), and phosphorus (P) cycling.

4. Evaluate the combined effects of multiple sustainable practices—including cover cropping, crop rotation, reduced tillage intensity, and alley cropping—on soil health and crop performance in a conventional cereal-based system.

By addressing these objectives, this thesis will contribute to a better understanding of how multiple cropping systems can be leveraged to support sustainable agricultural practices, promote soil conservation, and reduce the environmental footprint of crop production.

3. List of papers

The following papers are the result of research activities conducted in experimental fields located in Cesa (Tereto Living Lab, established for the PRIMA MA4SURE project in October 2021) and in Signa, within a detention basin designed for stormwater management. These activities were carried out at the Department of Agriculture, Food, Environment, and Forestry (DAGRI) of the University of Florence, during the period from October 2021 to September 2024. This research also benefited from scientific collaborations with the University of Seville and the University of Ljubljana, which enriched both the methodological approach and the interpretative scope and outcomes of the trials.

1. Pescatore, A., Vergari, D., Orlandini, S., Napoli, M. (2023). Agronomical management of detention basin. Italian Journal of Agrometeorology (1): 3-11. doi: 10.36253/ijam-2239

2. Pescatore, A., Delgado, A., Orlandini, S., Napoli M. (2025) Unveiling the impact of tillage radish on soil chemical, biological, and physical soil properties under reduced and conventional tillage. Soil and Tillage Research, 253, 106676. https://doi.org/10.1016/j.still.2025.106676

3. Pescatore, A., Delgado, A., Orlandini, S., Vergari, D., Napoli, M. (2025) Tillage Radish as Cover Crop Improves Soil Health Indicators Depending on Pedoclimatic Conditions. Soil Use and Management, 41(2), e70089. https://doi.org/10.1111/sum.70089

4. Pescatore, A., Mihelic, R., Orlandini, S., Napoli, M. Integrating multiple sustainable practices in Cropping Systems: A Field Study in the Mediterranean Region

   Under review in European Journal of Agronomy

4. First Paper

Agronomical management of detention basin

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4.1. Abstract

The importance of effective stormwater management through detention basin arrangement has become increasingly evident due to recurring extreme events in recent years. Limitations of the traditional detention basin include a reduced ability of basins to infiltrate water due to compacted soil and the carbon cost associated with the Diesel-powered tractors with lawn shredders. This study aims to compare six different agronomical management approaches for detention basins to improve the water storage capacity and the carbon sequestration potential, including the cultivation of crimson clover, white clover, tillage radish, and two mono-dicotyledonous mixes, against the conventional stable lawn-based approach. The trial was conducted in the detention basin in Castelletti (Firenze, Italy) for one growing season (2020/2021) according to a randomized complete block design with 9 replicates. Soil physical and chemical properties, as well as soil water storage capacity, were assessed to determine the feasibility of agronomical management for detention basins. Results indicated that the different treatments significantly influenced aboveground biomass production, soil organic carbon (SOC) stock, carbon sequestration potential, and water storage capacity. Specifically, crimson clover exhibited the highest aboveground biomass of around 6 t ha^{-1} among the treatments, while tillage radish demonstrated the greatest carbon sequestration potential (4.58 t CO_2 ha^{-1}), stable carbon stock in soil (1.14 t S-SOC ha^{-1}), as well as the highest potential for improving the water storage volume (389 m^3 ha^{-1}) in the topsoil (0-20 cm) of the detention basin. The findings suggested also that the sowing of different mono-dicotyledonous plant mix were poorly effective in improving carbon sequestration potential and water storage volume compared to conventional basin management. To sum up, this experiment has demonstrated that alternative agronomical management practices can enhance the capacity of detention basins to store carbon and stormwater. These results provide valuable insights for improving the sustainability and functionality of detention basins.

Keywords: carbon sequestration potential, stable soil organic carbon, soil water storage, detention basin, drainable porosity.

4.2. Introduction

The recurring extreme events of the last years have highlighted the importance of appropriate stormwater management. Excessive stormwater usually poses problems for rural communities and their livelihoods. The rate of soil impermeabilization, as well as the loss in water storage capacity of agricultural soils, have dramatically soared the water stream flow, determining the increase in soil erosion (Adobati and Garda, 2020). Management plans have been formulated by the European Environment Agency (EEA) to keep the water cycle as sustainable as possible (EEA, 2016). Different strategies accounted for in the best management practices (BPM), have been developed to reduce flooding risk, improve water quality, and recharge groundwater, such as the construction of swales, infiltration basins, retention basins, and detention basins (Davis et al., 2017; Lam et al., 2011). Those basins had been commonly known since ancient times, but with the increase in flooding events, their construction has recently gained attention. Although those ponds' general aim is to reduce stormwater peak flow of torrents and rivers, some distinctions have to be made.

Swales are ditches designed to collect, store, and reduce water runoff both in urban and rural environments, while improving the rate of stormwater that flows toward the groundwater (Sañudo-Fontaneda et al., 2020). Infiltration basins are ponds whose primary function is to improve the amount of water that infiltrates into the soil, hence their design has to facilitate the water flow from the ground surface to the groundwater (Di Lena et al., 2023). Retention basins are the most commonly designed structures for the mitigation of flooding events involving a permanent pool made with landscaped banks and surroundings (Acheampong et al., 2023). Detention basins are designed to temporarily store stormwater, but most of the time of the year they are free from water

(Sharior et al., 2019), therefore this lead to their putative use for agricultural purposes outside the rainy season.

Generally, detention basins allow the handling of a higher water volume than that of the usual riverbed; when the river flow is lowered, the basin-stocked water flows back to the river (Emerson et al., 2005). They could be used for managing and treating surface water runoff from impermeable surfaces such as roads (Griffiths, 2017). The conventional management of (dry) detention ponds consists of a soil bed where native plant species capable of tolerating periodic inundation are left to grow up and periodically mowed (Emmerling-DiNovo, 1995). Limitations of the traditional detention basin include a reduced ability of basins to infiltrate water due to compacted soil. One cause of this is that the soil at the bottom of these basins may become overly compacted during construction. Further, these basins are typically planted with turf grass that is shredded and mowed regularly 1-2 times a year to control the growth of above-ground biomass and the formation of shrubs. However, tractor traffic on lawns can have a significant impact on reducing the maximum root number and air-filled porosity in the upper 5 cm of soil, thereby decreasing the soil infiltration rate (Sveistrup and Haraldsen, 1997). Thus, a significant volume of water passes through these basins without the opportunity to infiltrate into the basin soil. Infiltration of precipitation and runoff water is an important green infrastructure goal, since infiltration recharges groundwater, decreases total runoff, and helps remove pollutants from the runoff water. Further, while lawns can function as "carbon sinks," their benefit is often outweighed by the carbon cost associated with their maintenance, specifically Diesel-powered tractors with lawn shredders.

The soil properties of detention basins can be improved by adopting alternative agronomical management while providing environmental and ecosystem services. For example, lawn management can be substituted by the cultivation of a specific crop that might enhance the soil water storing capacity. In this context, the soil in detention basins can be periodically tilled using a chisel plow and harrow to facilitate water accumulation but also to avoid the excessive soil compaction, that compromises detention basin functionality. In addition, the putative crop should produce both high aboveground and root biomass to increase carbon sequestration; previous properties could be satisfied with the sowing of different clover species, which usually occur in frequently flooded habitats (Huber et al., 2009).

However, few studies have been realized on the effect of tillage management on the soil properties of detention basins. Moreover, during the water storage, detention basins represent also a humid area, where some ecosystem functions could be achieved, such as wastewater treatment and the creation of wildlife habitats (Sharma et al., 2023).

The agronomical management of a detention basin could take into account that it is a periodically flooded soil; hence, some soil properties alterations should be observed. According to Schroer et al., (2018), the sediments transported by the water flow toward the detention basins are capable of increasing the carbon, nitrogen, and phosphorus concentration in soil. Accordingly, the basin could also support plant growth and reduce the carbon concentration in the atmosphere. In terms of nutrient load, the analyses of the water inflow revealed a significant amount of phosphorus and nitrogen that could be useful for plant growth (Wissler et al., 2020). In the same study, it was demonstrated that unmaintained detention basins were able to sequester higher amounts of carbon concerning maintained ones over 20 years; however, the maintenance they considered was only the turf grass mowing. Other authors reported an increase in soil particulate organic carbon (POM-C) concentration in a detention basin as a consequence of runoff water flow (Stanley, 1996).

On the other hand, no significant changes in organic matter (OM), carbon, and nitrogen concentration were observed in turf-grassed detention basins for the 0-5 and 15-20 soil layers (McPhillips et al., 2018).

Consequently, this work aimed to compare six different management of the detention basin to improve its capacity to stock carbon and store stormwater. Specifically, the sowing of different species of clover, the sowing of tillage radish, and the sowing of two crop mix termed Fascia Tampone and Rustico Dicotiledoni were compared to the conventional management of the detention basin, consisting in the growth of a stable lawn, which is periodically mowed. Some soil physical and chemical properties and soil water properties were measured for the different treatments to assess the feasible agronomical management of the detention basins, which are commonly considered marginal areas.

4.3. Materials and Methods

4.3.1. Experimental setup

The trial was carried out at the detention basin of Castelletti (Signa, Florence, Italy, 43° 47' 49" N, 11° 4' 51" E), from October 2020 to September 2021. The test site consisted of a surface of 4.2 ha that was subdivided into 15 adjacent watersheds around 2500 m^2 in size. The area was recovered with some projects that aim to restore traditional lowland agricultural hydraulic arrangement patterns and establish areas to promote biodiversity. The experimental design was a randomized completely block design, consisting of 15 blocks identified with the different treatments (3 blocks for treatment); for each block, 3 replicates were considered, hence a total of 9 replicates for treatment were obtained. According to the initial characterization, the soil was silt loam textured as the percentage of sand, silt, and clay were 21.6%, 53.8%, and 24.6%, respectively. The soil pH of the detention basin was neutral (7.10) with an average bulk density of 1.45 t m^{-3}.

Before the arrangement of the trial, the detention basin was managed with a stable lawn, and the soil was periodically tilled with a plow and harrow; before the sowing of different plants, the soil of the detention basin was plowed and harrowed using moldboard plow and disk harrow, respectively. Six different management of the detention basin were compared: the stable lawn (SL) was considered the control as it was the conventional management before the arrangement of the trial; crimson clover (CC; *Trifolium incarnatum* L.); white clover (WC; *Trifolium repens* L.); tillage radish (TR; *Raphanus sativus* L. var. Longipinnatus); a mono-dicotyledonous mix called OP-Rustico dicotiledoni (RD), a mono-dicotyledonous mix called OP-Fascia tampone (FT). The species description for each treatment is reported in Table 1. No fertilizers and pesticides were applied over the entire field. CC, FT, RD, TR, and WC were sowed at a seeding rate of 25, 35, 55, 18, and 25 kg seeds ha^{-1}, respectively. The TR root system is mainly composed of a taproot with only some fibrous lateral roots. RC and WC have both fibrous lateral roots and a taproot. SL is mainly composed of graminaceous species with fibrous roots, while FT and RD are mixtures comprising both graminaceous species with fibrous roots and leguminous species with both fibrous lateral roots and a taproot. The biomass collection for each treatment was carried out on May 21'. A sampler of 25 cm*25 cm was used to collect the aboveground biomass of the different treatments. The dry weight of the plant biomass was measured after oven drying at 70°C, until reaching constant weight.

Table 1: Description of the species for the different treatments (SL: stable lawn; CC: crimson clover; WC: white clover; TR: tillage radish, FT: OP-Fascia tampone; and RD: OP-Rustico dicotiledoni)

Herbaceous species in the different treatments					
SL	**CC**	**WC**	**TR**	**FT**	**RD**
Bellis perennis L.	*Trifolium incarnatum* L.	*Trifolium repens* L.	*Raphanus sativus* Var.Longpinnatus L.H.Bailey	*Achillea millefolium* L.	*Achillea millefolium* L.
Bromus hordeaceus L.				*Dactylis glomerata* L.	*Leucanthemum vulgare* Lam.
Cynodon dactylon L.				*Festuca rubra* L.	*Lotus corniculatus* L.
Lolium perenne L.				*Lolium arundinaceum* L.	*Medicago sativa* L.
Holcus lanatus L.				*Lolium perenne* L.	*Onobrychis viciifolia* Scop.
Hordeum murinum L.				*Lotus corniculatus* L.	*Phacelia tanacetifolia* Benth.
Plantago Media L.				*Onobrychis viciifolia* Scop.	*Plantago lanceolata* L.
Cichorium intybus L.				*Phacelia tanacetifolia* Benth.	*Salvia pratensis* L.
				Poa pratensis L.	*Silene vulgaris* Moench.
				Trifolium pratense L.	*Trifolium pratense* L.
				Trifolium repens L.	*Trifolium repens* L.

4.3.2. Fuel consumption for the different management

The main fuel-consuming activity was plowing at 30 cm depth because of the high energy requirement for moving a huge amount of soil (Table 2). Also, the preparation of the seedbed for the cultivation of CC, WC, FT, and RD represented a highly energy-consuming activity. The following activity that requires high-energy consumption was the soil disk harrowing, which was null for the conventional management of the detention basin. As regards the management of the aboveground biomass, all the treatments required two grass shredding per year, except the TR which requires only one per year.

Table 2: Diesel fuel consumption (L ha^{-1}) of the different management of detention basin (SL: stable lawn; CC: crimson clover; WC: white clover; TR: tillage radish, FT: OP-Fascia tampone; and RD: OP-Rustico dicotiledoni) for the different agronomical practices (plowing, disk harrowing, sowing, first and second shredding operations)

	Diesel fuel consumption per treatment (L ha^{-1})					
	SL	**CC**	**WC**	**TR**	**FT**	**RD**
Plowing (30 cm)		60	60		60	60
Disk Harrowing		33	33	33	33	33
Sowing		6	6	6	6	6
Shredding 1st time	25	25	25	25	25	25
Shredding 2nd time	25	25	25		25	25
Total fuel consumption	50	149	149	64	149	149

4.3.3. Soil sampling and analyses

The collection of soil samples was carried out according to the core sampling method; specifically, three samples for the plot were collected and mixed for 3 different soil depths (0-5, 5-10, 10-20 cm) both as disturbed and undisturbed samples. Disturbed samples were collected to determine some soil chemical properties, while soil physical properties were measured on undisturbed samples. The first soil sampling was carried out in August 2020 for the initial characterization of the soil, and after the soil tillage, the second sampling was performed (October ’20); in September’21 after the first growing season, the subsequent soil samples were collected.

The collected samples were air-dried and filtered by a 2-mm sieve. Soil Organic Carbon (SOC; g C kg^{-1}) was measured on disturbed soil samples using the CHNS elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Starting from the SOC it is possible to determine the amount of stable organic C by subtracting the Labile organic C from the SOC; labile organic C is obtained through the addition of particulate-organic carbon (POM-C; g C kg^{-1}) and Permanganate-Oxidable Carbon (POX-C; g C kg^{-1}). The determination of both POM-C and POX-C in soil was assessed through the methods proposed by Cambardella and Elliott, (1992) and Blair et al., (1995). Briefly, POM-C was measured by dissolving 10 g of soil into 30 mL of sodium hexametaphosphate (5 g L^{-1}). The dispersion was left for 15 h on a reciprocal shaker; then the dispersion was filtered through a 53-µm sieve. After various water rinsing, it was dried overnight at 50°C. The resulting dried sample was ground using a mortar and pestle and then subjected to the CHNS elemental analyzer. Regarding POX-C, a solution of $KMnO_4$ (52.625 g L^{-1}) was used for the oxidation of the soil sample. The soil containing 15 mg of SOC was dissolved in 25 mL of the $KMnO_4$ solution and left shaking for 1 h. Afterward, the tubes were centrifugated at 3500 rpm for 5 minutes and the resulting supernatants were diluted to 1:500. The absorbance of the sample was measured at 565 nm using a spectrophotometer. The bulk density (BD; t m^{-3}) of the detention basin for the different treatments was measured on undisturbed soil samples using cylinders of known volume. The SOC stock, the POM-C stock, and the POX-C stock of the 20 cm layer for the different treatments were calculated according to the following formulas:

$$\text{SOC}_{\text{Stock}} = \frac{(\text{SOC}_{\text{Sept'21}} * \text{BD} * 0.2) - (\text{SOC}_{\text{Oct'20}} * \text{BD} * 0.2)}{100000} \quad (1)$$

$$\text{POM-C}_{\text{Stock}} = \frac{(\text{POM}-\text{C}_{\text{Sept'21}} * \text{BD} * 0.2) - (POM - C_{\text{Oct'20}} * \text{BD} * 0.2)}{100000} \quad (2)$$

$$\text{POX-C}_{\text{Stock}} = \frac{(\text{POX}-\text{C}_{\text{Sept'21}} * \text{BD} * 0.2) - (\text{POX}-\text{C}_{\text{Oct'20}} * \text{BD} * 0.2)}{100000} \quad (3)$$

Where $\text{SOC}_{\text{Sept'21}}$ is the amount of organic C measured in September '21, BD is the bulk density measured in October '20, $\text{SOC}_{\text{Oct'20}}$ is the amount of organic C measured in October '20, $\text{POM-C}_{\text{Septs'21}}$ is the POM-C measured in September '21, $\text{POM-C}_{\text{Oct'20}}$ is the POM-C measured in October '20, $\text{POX-C}_{\text{Septs'21}}$ is the POX-C measured in September '21, $\text{POX-C}_{\text{Oct'20}}$ is the POX-C measured in October '20 and BD is the bulk density measured in October '20. The previous parameters were used to calculate the stable SOC stock (S-SOC; t ha^{-1}) as follows:

$$\text{S-SOC}_{\text{stock}} = \text{SOC}_{\text{stock}} - (\text{POM} - \text{C}_{\text{stock}} + \text{POX} - \text{C}_{\text{stock}}) \quad (4)$$

The calculation of the drainable porosity (%) for the determination of the water storage volume (WSV; m^3 ha^{-1}) for the different treatments was assessed through the soil water retention curve. Specifically, the water percentage at field capacity was measured through the water retention curve using Richard's plate apparatus (Richards and Fireman, 1943). The estimation of the total porosity was calculated as follows:

$$\text{Total porosity [\%]}=\left[1-\left(\frac{BD\left[t\ ha^{-1}\right]}{2.65\ [t\ ha^{-1}]}\right)\right]*100 \qquad (5)$$

Where BD is the bulk density measured, and 2.65 is the estimated real density. Accordingly, the drainable porosity was calculated as the difference between the total soil porosity and the water percentage in the soil at field capacity. Lastly, the WSV was calculated as the amount of drainable porosity in the topsoil (20 cm).

4.3.4. Statistical analysis

The comparison among the means of different treatments for each variable was assessed according to the one-way ANOVA. The parameters which resulted significantly were compared through a post-hoc Tukey's Test.

4.4. Results and Discussion

4.4.1. Weather description

The climate of the area was Mediterranean with an average annual rainfall and a mean annual temperature of 872 mm and 15.25°C, respectively (Figure 1). The annual rainfall amount during the 2020/2021 growing season was similar to the long-term average (2001–2020), with no notable variation observed. However, by comparing the monthly data, it was observed that rainfall that occurred during the 2020/2021 growing season exceeded the long-term average in October, December, and January, leading to the flooding of the detention basin for 4 days in December and January. On the other hand, the area experienced a low rainfall amount concerning the long-term average in November 2020 and March 2021. The temperature pattern of the 2020/2021 growing season was quite similar to the long-term trend of temperature, except for the spring months when the average monthly temperature of the 2020/2021 growing season was lower than those reported for the long-term.

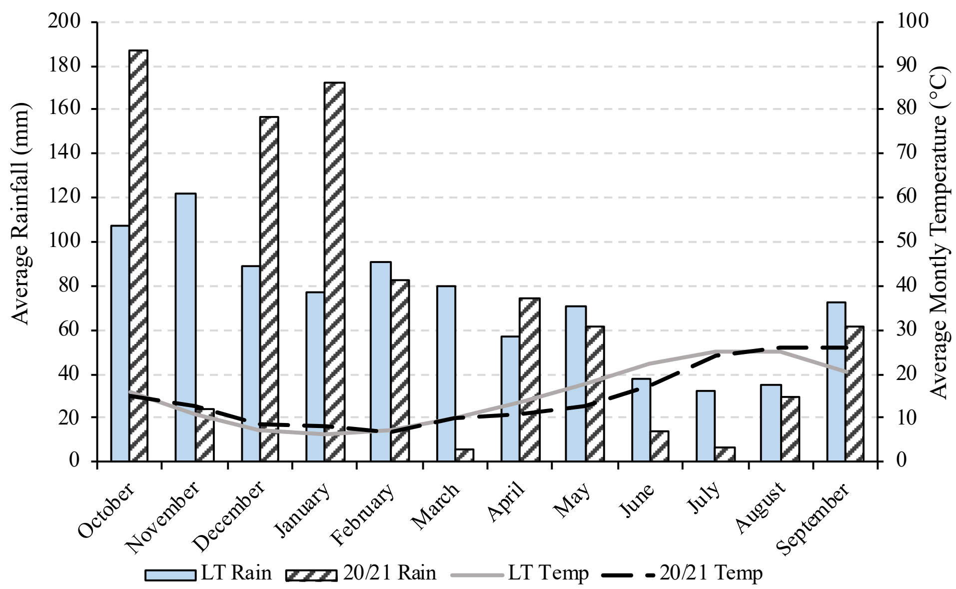

Figure 1: Walter-Lieth diagram of the climatic conditions at Castelletti, Florence, Italy. Light blue and striped histograms indicate the rainfall amount of the long-term (2001-2020) and 2020/2021 growing season, respectively; gray and dashed lines show the temperature trend of the Long-Term and 2020/2021 growing season, respectively.

4.4.2. Aboveground Biomass yield

The different treatments significantly affected the amount of aboveground biomass (AG-y) produced over the detention basin (Figure 2). The AG-y produced by the different treatments ranged between 2.71 and 6.04 t ha^{-1} of DW. Specifically, CC highlighted the best performance, accounting for 6.04 t ha^{-1} of AG-y. Indeed, Knight, (1985) reported that CC can successfully and rapidly grow in a wide range of climatic and soil conditions. The observed yield of CC was similar to that reported in SARE Outreach, (2007), indicating that crimson clover can reach 7 t ha^{-1} DW in good growing conditions. The AG-y of WC was significantly lower than that of CC, resulting in 34.2% lighter than WC; in fact, WC AG-y was more negatively affected by soil flooding than CC was. The WC susceptibility to flooding was also been described by Huber et al., (2009). The AG-y in SL was significantly lower than that measured in WC but not significantly different from CC and TR. The AG-y in SL, mainly composed of graminaceous plants including common ryegrass, was consistent with the average annual AG-y value of approximately 5 t ha^{-1} reported by several authors for common ryegrass (Vinther, 2006). The AG-y value measured in TR was 3.43 t ha^{-1} and was consistent with that reported by Cottney et al., (2022) for the tillage radish sowed in September. The aboveground biomass of the two herbaceous mixes, FT and RD, were significantly lower compared to the other treatments.

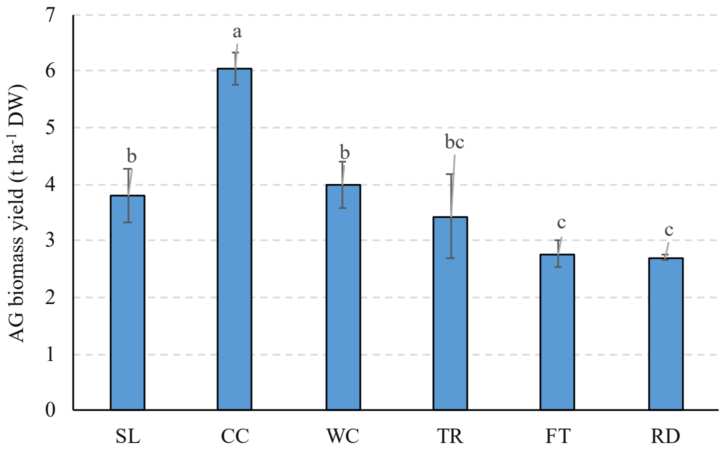

Figure 2: Aboveground biomass yield (AG-y; t ha^{-1} Dry weight) for the different management of detention basin (SL: stable lawn; CC: crimson clover; WC: white clover; TR: tillage radish, FT: OP-Fascia tampone; and RD: OP-Rustico dicotiledoni). Error bars represent the standard deviation (n=9). The letters indicate significant differences between the treatments according to the post-hoc Tukey's test.

4.4.3. Carbon Stocking capacity

After 1 year, the SOC stock ranged between -0.08 t ha^{-1} in FT to 1.25 t ha^{-1} in TR (Table 2). The TR treatment produce the significantly highest increase in SOCstock, followed by CC and WC. The SOCstock variation in SL was not significant, while a not significant negative variation was observed in FT and RD. The labile C stock ranged between -0.08 t ha^{-1} in FT to 0.12 t ha^{-1} in WC. However, no significant differences in labile C stock were detected between the six treatments. The S-SOC stock ranged between -0.05 t ha^{-1} in FT to 1.14 t ha^{-1} in TR. The TR treatment produce the significantly highest increase in S-SOC stock, followed in decreasing order by CC and then WC. Further, the S-SOC stock variation in SL, FT, and RD was not significant. These values are consistent with Franzluebbers et al., (2012) who observed that after the conversion of an arable cropping system into perennial grassland the rate of C accumulation down to a depth of 20 cm has an initial value of 0.8 t ha^{-1} y^{-1}. Probably for mixes containing grasses as in FT and RD, the time required to recover the oxidized carbon through soil tillage is longer than a single year of cultivation. As reported by Li et al., (2020) and Liu et al., (2015) the tap-root system may have a higher impact on increasing SOCstock in soil than the fibrous roots. Therefore, the differences between the treatments can be attributable to the different root systems of the plant species in the six treatments.

Table 3: Changes in SOC stock, Labile C stock, and Stable C stock (S-SOC) for the different treatments (SL: stable lawn; CC: crimson clover; WC: white clover; TR: tillage radish, FT: OP-Fascia tampone; and RD: OP-Rustico dicotiledoni). Standard deviation values are reported in brackets. The letters indicate significant differences among the treatments according to Tukey's test ($p<0.05$)

Treatments	SOC stock t ha^{-1}	Labile C stock t ha^{-1}	S-SOC stock t ha^{-1}
SL	0.08 (0.18) c	0.07 (0.1)	0.01 (0.13) d
CC	0.59 (0.12) b	0.05 (0.12)	0.54 (0.14) b
WC	0.47 (0.04) b	0.12 (0.06)	0.35 (0.08) c
TR	1.25 (0.56) a	0.11 (0.26)	1.14 (0.21) a
FT	-0.08 (0.03) c	-0.08 (0.1)	0 (0.09) d
RD	-0.01 (0.02) c	0.04 (0.07)	-0.05 (0.08) d

4.4.4. Carbon sequestration

The annual amount of fixed CO_2 ranged between -0.29 and 4.58 t ha^{-1}, respectively in FT and TR, when considering the whole SOC_{stock} (Figure 3). According to our results, TR was the best treatment in terms of annual carbon sequestration potential, resulting in significantly higher carbon sequestration of 53.65% and 62.43% concerning CC and WC, respectively. The annual carbon sequestration rate SL (0.30 t CO_2 $ha^{-1}y^{-1}$) was significantly lower than that calculated for CC and WC. Lastly, negative values of carbon sequestration were calculated for RD and FT, indicating that the carbon that was released into the atmosphere by these treatments was higher than that incorporated in the soil. Specifically, FT showed a significantly lower carbon sequestration potential than RD. The amount of annual CO_2 sequestered by SL was in agreement with that reported by Yang et al. (2019) for topsoil (20 cm) with 8 species making up the grassland system. However, the potential carbon sequestration of the same author for a single species system was lower than we have observed. On the other hand, our results were not consistent with the estimation of carbon balance for Western Europe by Dondini et al. (2023) for an unimproved grassland system of about 4.40 t CO_2 ha^{-1} y^{-1}. However, the value of carbon balance these authors estimated for unimproved grassland was quite similar to the value of the carbon sequestration potential of TR.

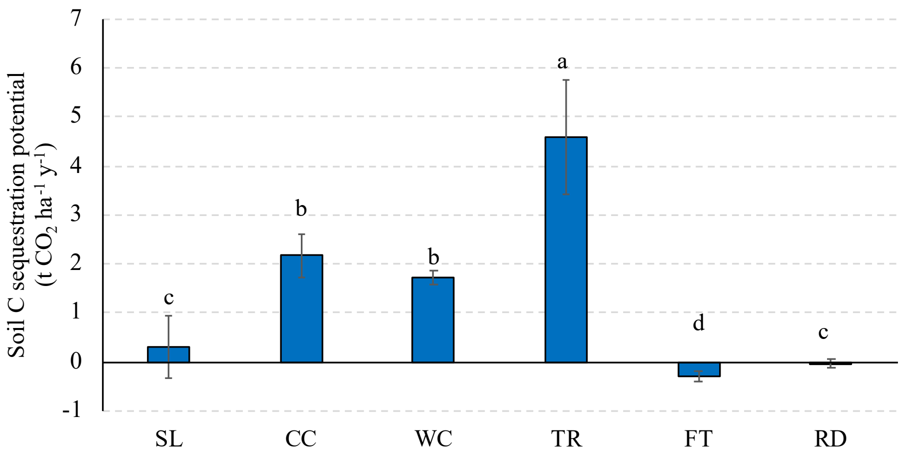

Figure 3: Annual Carbon sequestration rate (t CO_2 $ha^{-1}y^{-1}$) for the different management of detention basin (SL: stable lawn; CC: crimson clover; WC: white clover; TR: tillage radish, FT: OP-Fascia tampone; and RD: OP-Rustico dicotiledoni). Error bars represent the standard error (n=9). The letters indicate significant differences between the treatments according to Tukey's test (p<0.05).

4.4.5. Water Storage Volume

The correct management of the detention basin was also assessed through the calculation of its capacity to store water (Figure 4). Our results highlighted significant differences in the WSV among the different treatments, ranging from 258 to 389 m^3 ha^{-1} for RD and TR, respectively. In particular, the highest WSV value was detected in TR, which was significantly higher than CC and WC by 6% and 8% respectively; the average WSV value of CC (364 m^3 ha^{-1}) was significantly higher than that of WC (356 m^3 ha^{-1}). Lastly, the lowest average WSV values were detected in RD, which were not significantly different from that detected in SL and RT, indicating that the behavior of FT and RD in determining the WSV was very similar to that of SL. The WSV values of the latter were quite similar to those reported by Zhu et al. (2022) for the topsoil (20 cm) of mountain grassland. Similarly, the WSV values observed by Otremba et al., (2021) were around 280 m^3 ha^{-1} considering the 0-35 soil layer after one year of alfalfa and orchard grass cultivation. On the other hand, similar results for clover WSV values were obtained by Fang et al., (2023). The good performance in terms of WSV obtained by TR could be determined by the wide holes that taproot leaves in the soil (White and Weil, 2011).

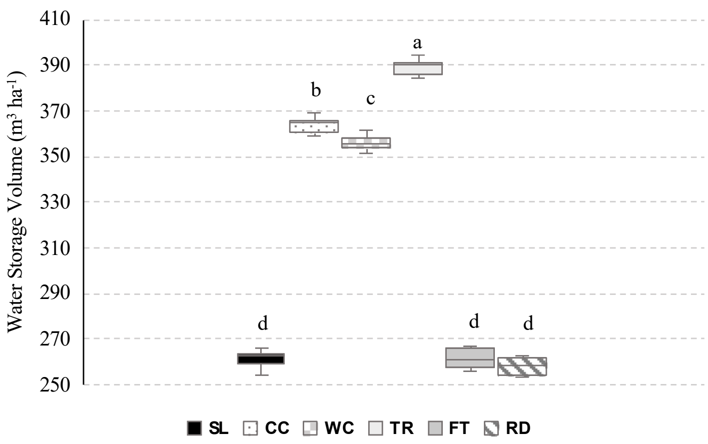

Figure 4: Soil Water Storage Volume (m^3 ha^{-1}) for the different management of detention basin (SL: stable lawn; CC: crimson clover; WC: white clover; TR: tillage radish, FT: OP-Fascia tampone; and RD: OP-Rustico dicotiledoni). Error bars represent the standard error (n=9). The letters indicate significant differences between the treatments according to Tukey's test.

4.5. Conclusions

The different plants in the agronomic treatments were rapidly adapted to the detention basin conditions, especially the crimson clover that produced the highest aboveground biomass yield. Regarding the increase of soil organic carbon, the best performance was obtained by the tillage radish, which could represent a good strategy for increasing soil stable organic carbon. Likewise, tillage radish was also enormously effective in increasing the water storage capacity of the detention basin, followed by the crimson clover and white clover. In summary, the results emphasize the importance of plant selection for the effective management of detention basins. Crimson clover and tillage radish emerged as promising options for maximizing aboveground biomass production, improving soil organic carbon stock, enhancing carbon sequestration potential, and increasing water storage capacity. The findings provided valuable insights for the design and implementation of sustainable and efficient detention basin management strategies, highlighting the role of specific plant species in achieving desired outcomes. Further research and long-term monitoring are needed to fully understand the dynamics and long-term effects of these treatments on the detention basin dynamics.

4.6. Acknowledgments

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5. Second Paper

Unveiling the impact of tillage radish on soil physicochemical properties under reduced and conventional tillage

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5.1. Abstract

The timespan between the crop harvesting and the subsequent crop sowing presents an opportunity to improve soil properties through the sowing of cover crops (CCs). However, the short-term effects of specific CCs, such as tillage radish (TR), on soil properties under different tillage systems remain insufficiently explored, particularly in the European context. This study examined the short-term effects of TR sowing on soil properties under reduced and conventional tillage systems. Results demonstrated that TR biomass yield and phosphorus uptake were consistently higher under conventional tillage compared to reduced tillage. The highest increase in soil organic carbon across soil depths was observed in plots sown with TR under conventional tillage, which was 60–75% greater than that under reduced tillage. TR increased particulate organic carbon by 16.5 mg C kg^{-1} in reduced tillage and 65.2 mg C kg^{-1} in conventional tillage compared to fallow. The sowing of TR under conventional tillage also influenced soil Olsen phosphorus, with a threefold increase in the upper soil depth compared to reduced tillage. Microbial biomass carbon, phosphorous, and nitrogen showed similar trends with respect to their chemical pools, with higher values in TR under conventional tillage. Regarding the soil physical properties, bulk density was higher under reduced tillage, particularly in deeper layers, compared to under conventional tillage. Overall, the sowing of TR provided short-term soil benefits on some soil properties, especially under conventional tillage. Further research is needed to evaluate its performance and benefits across diverse soils and cropping conditions.

Keywords: Cover crops, soil health, ecosystem services, microbial biomass, fallow substitution

5.2. Introduction

In recent years, the use of cover crops (CCs) has gained prominence as an essential practice for preserving and enhancing soil health while providing ecosystem services (Yousefi et al., 2024; Garba et al., 2024). Cover crops and reduced tillage have been proposed as sustainable alternatives to conventional cropping systems, which typically involve bare soils between crops and intensive tillage, in order to mitigate their impact on soil health and the environment. Such conventional practices often contribute to soil degradation, nutrient losses, and negative environmental impacts. CCs, which are typically grouped into legumes, non-legumes, and grasses (Koudahe et al. 2022), offer numerous agronomic and environmental benefits, including reducing erosion, reducing leaching, improving C storage in soil, increasing biological activity, and recycling and providing nutrients to main crops (Adetunji et al. 2020; Wittwer and van der Heijden, 2020).

The importance of CCs is underscored by policies such as the European Union's Common Agricultural Policy (CAP) 2023-2027, which actively encourages CC sowing and reduced tillage practices to achieve more sustainable farming systems (Fendrich et al. 2023). Most of CCs trials have been conducted in North and South America, where the use of CCs is widely accepted by farmers (Zou et al. 2022). Cover crops, together with reduced tillage intensity and increased diversification in crop rotations, are the pillars of conservation agriculture (CA).Despite their well-documented benefits, Fendrich et al. (2023) highlighted that the adoption of CCs cultivation in Europe is limited to a handful of countries due to political, economic, and agronomic reasons. One of the main agronomic reasons is the uncertainty about the benefits of CCs (Kathage et al. 2022); therefore, further practical evidence is needed to provide convincing arguments for its adoption.

Addressing these concerns requires selecting CC species that are cost-effective, require minimal management, and demonstrate clear agronomic benefits. Among potential CC species, tillage radish (TR; *Raphanus sativus* var. longipinnatus L.H.Bailey), a member of the Brassicaceae family, has emerged as a promising option. Its deep-rooting system can alleviate soil compaction by penetrating compacted layers, thereby improving soil porosity, water infiltration, and the rooting environment for subsequent crops, presenting the characteristics suggested by Koehler-Cole et al. (2023) that make it a suitable choice as a CC. The integration of TR with reduced tillage has the potential to enhance soil health after many years of conventional tillage (Kaur et al. 2024; Acharya et al. 2024). In addition, TR has rapidly decomposing residues, making it well-suited to align with the management needs and timing of the next cash crop (Koudahe et al. 2022).

Among the key agronomic benefits of TR is its ability to increase the availability of **phosphorus (P)** in soils. The low P availability has been recognized as a limiting factor for crop production across many regions of the world (Mancini et al. 2024; Recena et al. 2022). Like other members of the Brassicaceae family, TR can modify the rhizosphere by exuding organic acids and altering soil pH, which facilitates the solubilization of otherwise unavailable P forms (Gieske et al. 2016). Previous studies have, demonstrated that TR improves P availability in P-rich soils (White and Weil, 2011), and enhances P uptake by subsequent crops, such as ryegrass, even in P-deficient soils (Hansen et al. 2022). Furthermore, TR effectively captures nitrogen (N) from deeper soil layers, reducing nutrient losses via leaching thus contributing to reducing losses (Dean and Weil, 2009).

In addition to nutrient cycling, TR offers significant contributions to soil organic carbon (SOC) dynamics. The unharvested biomass of TR can increase soil carbon inputs (Derrien et al. 2023), with some studies showing significant increases in the total SOC stock in the 0-45 cm soil under reduced tillage systems combined with TR, compared to conventional tillage or bare soil management (Besen et al. 2024). On the other hand, Sartori et al. (2022) found no significant effect of TR and minimum tillage on total SOC. While long-term effects on SOC fractions remain unclear, short-term increases in oxidizable carbon fractions, such as permanganate oxidizable carbon (POX-C), have been observed following TR crop by Wang et al. (2017). These findings underscore the potential of TR to contribute to soil carbon sequestration and improve soil health parameters in the short term.

From a practical standpoint, TR offers advantages in terms of ease of management. In regions with freezing winters, TR can be terminated naturally through winter-kill, eliminating the need for mechanical or chemical termination and reducing associated costs (Tadiello et al., 2022). Furthermore, its adaptability to various pedoclimatic conditions makes TR a versatile choice for diverse European cropping systems (Koehler-Cole et al., 2023).

Despite these promising attributes, research on TR remains relatively limited, particularly in the European context. Further studies are required to fully evaluate its effects on soil chemical, biological, and physical properties across different management systems and climatic conditions. Blanco-Canqui (2023), emphasized the need to investigate the effects of individual CC species on different SOC fractions under contrasting cropping systems and pedoclimatic conditions. This research gap is particularly relevant for tillage radish in the European context. However, further studies are required to understand how tillage radish biomass interacts with soil properties and affects different SOC fractions, especially in diverse environmental and management scenarios.

It was hypothesized that substituting the bare soil period between main crops with TR as a CC will increase P availability for the subsequent crop, enhance short-term C storage, and positively influence other chemical, biological, and physical soil properties. Therefore, this study aims to investigate TR growth under conventional and reduced tillage and its impact on a set of relevant chemical, biological, and physical soil properties, compared to traditional bare soil management.

5.3. Materials and methods

5.3.1. Site description

The experiment was carried out over two consecutive growing seasons (2022-2023 and 2023-2024, referred to as GS1 and GS2) under rainfed condition at the Tereto Living Lab (CS; Marciano della Chiana, Arezzo, Italy; 43.3179 N, 11.7952 E, 246 m asl). The soil at the CS site is a typical Vertic Calciustepts (Soil Survey Staff, 2014) with a clay loam texture. Soil properties, including Olsen P levels, which are below the threshold value for fertilizer response (Recena et al., 2022), are detailed in Table 1.

According to the Köppen classification, CS has a hot-summer Mediterranean climate (Cfa), characterized by dry and hot summers and most rainfall in autumn. Cumulative rainfall from August to January was 560 mm for GS1 and 250 mm for GS2, compared to the long-term average of 430 mm (1991-2020). Average temperatures during this period were 14.2°C in GS1 and 14.4°C in GS2, both higher than the long-term average of 12.5°C.

Table 1 Soil properties determined before the sowing of the tillage radish at the Tereto Living Lab (CS) for the upper 15 cm soil layer

Soil property	Value ± SD
Sand (%)	32.82 ± 1.81
Silt (%)	32.24 ± 0.94
Clay (%)	34.94 ± 2.05
pH_w (1:5)	8.20 ± 0.03
EC (mS cm^{-1})	0.20 ± 0.01
CEC (meq 100 g^{-1})	28.97 ± 1.44
$CaCO_3$ (%)	5.62 ± 0.17

Soil Organic Carbon (%)	1.00 ± 0.55
Total Nitrogen (%)	0.10 ± 0.06
Olsen P (mg P kg^{-1})	7.90 ± 0.56

pH_w= pH in 1:5 soil water/extract; CEC=cation exchange capacity; EC= electrical conductivity in 1:2.5 soil water/extract

5.3.2. Field History

The field had a history of field crop cultivation with conventional tillage. Since 2015, a rotation of sunflower (*Helianthus annuus* L.) and durum wheat (*Triticum turgidum* subsp. Durum) was followed, with the soil left fallow from the harvest of winter cereals in July until sunflower sowing in March. Prior to the establishment of the current trial in 2021, all the field was cultivated under conventional tillage involving moldboard ploughing to 30 cm, followed by disc harrowing to prepare the seedbed. Starting in 2021, part of the field continued under conventional tillage, while the other half was managed with reduced tillage, consisting of chisel ploughing to 30 cm followed by disc harrowing to prepare the seedbed. During the GS1, the preceding crop was winter wheat (*Triticum aestivum* L.), while in GS2 it was spring barley (*Hordeum vulgare* L.).

5.3.3. Experimental design and crop management

The experiment tested two ground cover (GC) management practices: cover crop (CC) and no cover crop (nCC) (Figure 1). Trials were carried out on both the part of the field managed with conventional tillage (moldboard ploughing at 30 cm) and the part managed with reduced tillage (chisel ploughed at 30 cm). After the primary tillage, disk harrow was used over both parts to break the clods and prepare the seedbed. The GC treatments were arranged as a split-plot design with three blocks each containing 6 replicates, resulting in a total of 18 replicates per treatment. Plot measured 15 × 15 m

(225 m^2). In CC plots, TR was sown on September 14 and 16 for GS1 and GS2, respectively, at a rate of 18 kg of seed ha^{-1}. Immediately after TR sowing, a spring tine harrow was used in all plots to kill emerged weeds and to improve seed-to-soil contact in CC plots. During both GSs, no fertilizers or pesticides were applied; however, Glyphosate (2 L ha^{-1}) was applied to all plots in late February to terminate TR.

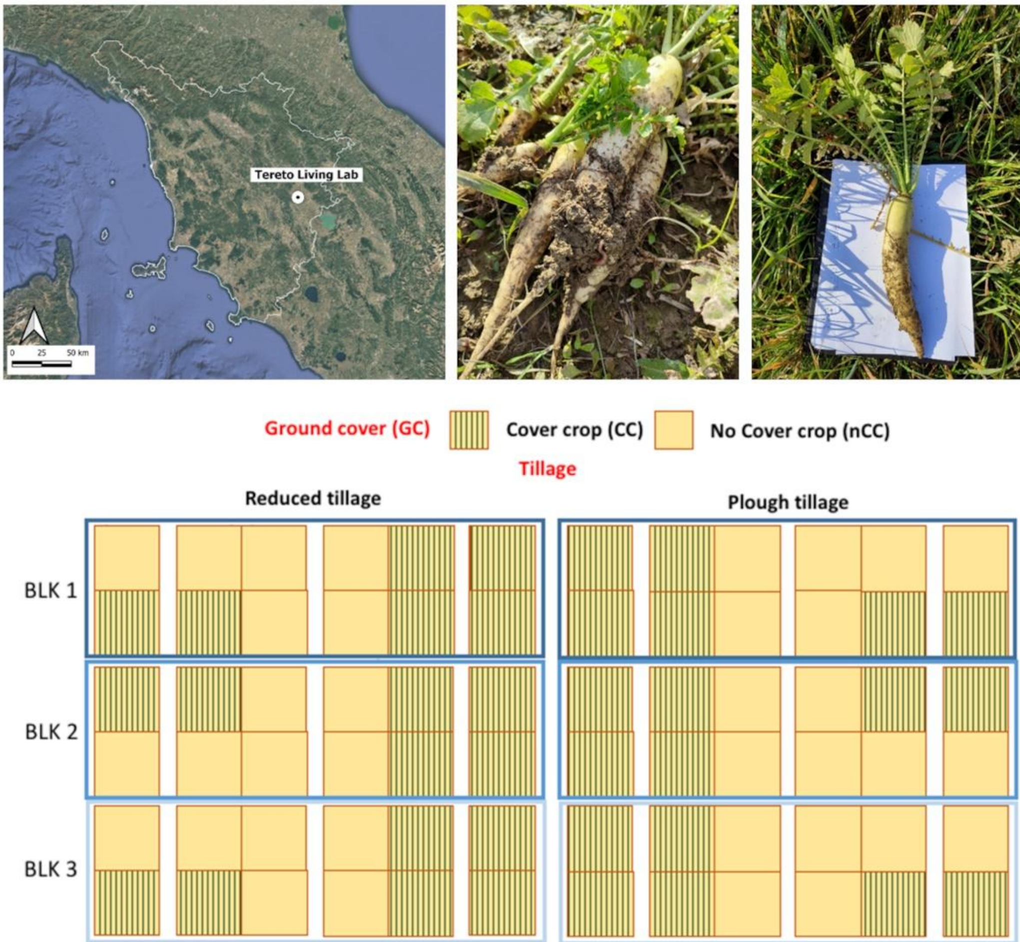

Figure 1: Experimental design at the Tereto Living Lab with BLK standing for blocks.

5.3.4. Biomass and soil sampling

Tillage radish biomass was sampled in late January in both GSs, with aboveground and taproot material collected from two randomly selected 0.25-m^2 areas per plot. Samples were oven-dried at

60 °C for 48 h until constant weight was achieved. Then, samples were weighed to estimate the TR's aboveground (Ab-Y) and taproot (Tap-Y) biomass of TR. The P concentration in plant tissues was determined by ashing 0.4 g of dried samples in a muffle furnace at 550°C for 5 hours. Afterward, the ash was dissolved in 40 mL of 0.3 M HCl and filtered through Whatman no. 42 filter paper. The P concentration in the filtrate was measured using the vanadomolybdophosphoric acid method (Kuo, 1996). Subsequently, P uptake by aboveground (P-upt Ab; kg P ha^{-1}) and taproot (P-upt Tap; kg P ha^{-1}) of TR was calculated as the product of the P concentration and biomass in respective biomasses.

Further, all plots (including non-cover crop plots) were sampled to assess aboveground weed biomass using the same procedure applied to TR. However, due to late-season disk harrowing and low autumn-winter temperatures, weed development was minimal, with no statistical differences detected between treatments. Given these conditions, weed biomass values were not included in further analyses, as they were negligible compared to TR biomass.

Soil samples were collected before TR sowing (September) and before TR termination (late January) at soil depths (SDe) of 0-5 cm, 5-10 cm, and 10-20 cm using bucket augers. Five subsamples per plot were composited for each soil depth. Samples for microbial analysis were stored moist at 4°C, while those for chemical analysis were air-dried and sieved to 2 mm. For the determination of soil bulk density (BD), three undisturbed soil core samples were collected at each soil depth using stainless steel cylinders (diameter: 53 mm, height: 50 mm). The sampling depth for BD determination was extended to 20–30 cm to ensure consistency with the depth of tillage operations.

5.3.5. Soil analyses

Soil organic carbon and total N were determined using a CHN analyser (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA). Before the analysis, the soil was ground using a planetary ball mill (Pulverisette 6, Fritsch, Idar-Oberstein, Gemany) (460 rpm; 2 min). The determination of Olsen P was performed according to the Olsen method (Olsen et al. 1954). Particulate organic carbon (POM-C) and permanganate-oxidable carbon (POX-C) were determined following methods by Cambardella and Elliot (1992) and Weil et al. (2003), respectively. Different products were tested to create a standard solution to generate a calibration curve for POX-C determination. Finally, L-Ascorbic acid was selected as the standard yielding results consistent with those reported by Woodings and Margenot (2023).

Microbial biomass carbon (MBC), microbial biomass phosphorus (MBP), and microbial biomass nitrogen (MBN) were determined by the fumigation-extraction method (Brookes et al. 1985; Vance et. al. 1987). For each composite sample, 20 g were weighed, stored in glass bakers, and fumigated, while other 20 g of the same sample were not fumigated. Fumigated and non-fumigated samples were extracted with 0.5 M K_2SO_4 for MBC and MBN, and with 0.5 M $NaHCO_3$ for MBP. The CHN analyser was used to determine C and N in the extracts for MBC and MBN, while P in the extracts for MBP was determined colorimetrically (Kuo, 1996). Correction factors (k_C) for MBC, MBP, and MBN were set at 0.38, 0.40, and 0.54, respectively.

Soil bulk density (BD) values were used to estimate the degree of compactness (DC) for the different treatments according to Keller and Håkansson, (2010). The reference BD (BD_r) was estimated using the soil textural classes and organic matter (OM) content as reported by Keller and Håkansson, (2010):

$$BD_r = 1.308 + 0.0119clay + 0.0103sand - 0.00018clay^2 - 0.00008sand^2 - 0.00062siltOM - 0.00059sandOM$$

Then, DC was calculated as follows:

$$DC = \frac{BD_m}{BD_r}$$

Where BD_m is the measured BD.

5.3.6. Statistical analysis

Statistical analyses were performed using the R environment (R Core Team, 2021). The assumption of normal distribution was tested by the Shapiro Wilk test. If the data did not meet the normality criteria, the Box-Cox power transformation from the MASS package in RStudio was applied (Venables and Ripley, 2002). Then, ANOVA was performed to evaluate the differences in TR parameters (Ab-Y; Tap-Y; P-upt Ab; P-upt Tap) between the two growing seasons (GS1 vs. GS2) and between the two tillage systems (reduced vs. conventional tillage), as well as their interactions, using the following model:

$$TR\ parameter = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \epsilon$$

Where: X_1 and X_2 represent the treatments GS, Tillage; β_1X_1 represent the main effect of GS; β_1X_2 represent the main effect of tillage; $\beta_{12}X_1X_2$ captures the interaction effect between GS and Till; β_0 represents the intercept, and ϵ is the error term.

As regards the soil parameters, the data collected at the end of the TR GSs were subtracted to the initial values just before the sowing of TR for each GS. Therefore, the soil data are expressed in terms of differentials (Δ) between post- and pre-sowing values for each GS. Before the analysis, the differential data were submitted to Shapiro Wilk test; when the normal distribution was not meet, the data were transformed by Box-Cox power transformation. Then, ANOVA was used to evaluate the effect of Till, GC, GS, SDe, and their interactions on the soil parameters in CS using the following model:

$$Soil\,parameter = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i<j} \beta_{ij} X_i X_j + \sum_{i<j<k} \beta_{ijk} X_i X_j X_k + \beta_{1234} X_1 X_2 X_3 X_4 + \epsilon$$

Where: X_3, and X_4, represent the treatments GC, and SDe respectively; $\beta_i X_i$ represent the main effect for each individual factor; $\beta_{ij} X_i X_j$ represent the two-way interactions for each pair of factors; $\beta_{ijk} X_i X_j X_k$ represent the three-way interactions for each combination of three factors; $\beta_{1234} X_1 X_2 X_3 X_4$ represent the four-way interactions involving all four factors.

A mixed model was used for the analysis of soil physical parameters (BD and DC). In particular, Till, GC, and SDe were considered as fixed factors. The data of BD and DC were divided depending on sampling time (Samp): before sowing of TR (PRE) and after termination of TR (POST). Afterward, the following model was developed:

$$Soil\,phys\,par. = \beta_0 + \sum_{i=2}^{5} \beta_i X_i + \sum_{i<j} \beta_{ij} X_i X_j + \sum_{i<j<k} \beta_{ijk} X_i X_j X_k + \beta_{2345} X_2 X_3 X_4 X_5 + \epsilon$$

Where: X_5 represents the new Samp factor.

When the ANOVA results indicated a significant effect of the factors on the variables, a Tukey's post-hoc test was performed. For significant interactions between factors, we applied a simple effects analysis to assess how each factor influenced the response variable within specific conditions of the interacting factor. This approach preserves interaction structure, providing clearer biological interpretation compared to all-against-all comparisons. Given the study's objectives, this method ensures meaningful treatment evaluation while maintaining result interpretability. The percentage of explained variance for each factor was determined by calculating its contribution relative to the total variance explained by the model. This was assessed using the sum of squares for each factor, considering all main effects and interactions while excluding the residual variance. The purpose of this analysis was to quantify the relative importance of each factor in explaining the variability of the dependent variable and to identify the most influential factors affecting the observed outcomes.

5.4. Results and Discussion

5.4.1. Dry matter accumulation and phosphorus uptake in tillage radish

The interaction between growing season (GS) and tillage system (Till) significantly influenced dry matter accumulation and phosphorus (P) uptake by tillage radish (TR) (Table 2). Specifically, this interaction significantly affected TR's biomass yield (Ab-Y), aboveground phosphorus uptake (P-upt Ab), and taproot phosphorus uptake (P-upt Tap).

The Ab-Y was significantly higher under conventional tillage than under reduced tillage in both GSs (Figure 2a). The reduced Ab-Y under reduced tillage likely reflects less soil loosening and residue incorporation due to the absence of moldboard ploughing, limiting early root establishment and nutrient and water access. Blanco-Canqui and Lal (2009) highlighted that reduced tillage slows residue decomposition, delaying nutrient availability, while Giller et al. (2009) suggested that reduced tillage can constrain root growth and crop performance, particularly without fertilization, as was also the case in our study. The reductions in Ab-Y under reduced tillage are consistent with findings by Yagioka et al. (2014), who observed that, in the absence of nitrogen fertilization, aboveground biomass of daikon radish was lower under reduced tillage than in conventional tillage.

Under reduced tillage, the value of Ab-Y in GS2 was approximately 89% lower than in GS1, whereas under conventional tillage, the reduction was 84.4%. This decline in Ab-Y during GS2 can likely be attributed to differences in weather conditions, particularly lower cumulative rainfall from August to January compared to GS1 (Supplementary Table 1). Reduced precipitation may have limited early radish establishment and aboveground biomass accumulation, as moisture availability is a key factor influencing cover crop development.

Table 2. Mean values (± standard error) and ANOVA results for tillage radish aboveground biomass yield (Ab-Y), taproot biomass yield (Tap-Y), aboveground phosphorus uptake (P-upt Ab), and taproot phosphorus uptake (P-upt Tap) under different treatments. Significant differences are indicated by *** ($p < 0.001$), ** ($p < 0.01$), and "ns" (not significant). Different letters indicate significant differences between treatments.

Variability sources	df	Ab-Y		Tap-Y		P-upt Ab		P-upt Tap	
		t ha^{-1}	sig	t ha^{-1}	sig	kg P ha^{-1}	sig	kg P ha^{-1}	sig
Main effect									
Till	1		***		***		***		***
Reduced		0.63 (0.09)	b	0.51 (0.03)	b	2.4 (0.34)	b	2.31 (0.15)	b
Conventional		1.98 (0.26)	a	1.83 (0.09)	a	9.27 (1.22)	a	8.38 (0.53)	a
GS	1		***		**		***		***
GS1		2.28 (0.21)	a	1.02 (0.11)	b	10 (1.12)	a	4.13 (0.45)	b
GS2		0.33 (0.07)	b	1.31 (0.15)	a	1.67 (0.26)	b	6.56 (0.81)	a
Interaction									
GS x Till	1		***		ns		***		**

Till: Tillage system (reduced vs conventional); GS: Growing Seasons (GS1 vs GS2)

Table S1. Monthly cumulative rainfall (mm) and average temperature (°C) recorded during the two growing seasons (GS1 and GS2) compared to the long-term climatic average (1991–2020). Values in parentheses represent standard deviations of the long-term data.

Month	Long term (1991-2020)		GS1		GS2	
	Cumulate rainfall (mm)	Average Temperature (°C)	Cumulate rainfall (mm)	Average Temperature (°C)	Cumulate rainfall (mm)	Average Temperature (°C)
August	42.3 (5.6)	23 (0.2)	68	24.3	28.4	23.6
September	68.8 (7.1)	18.6 (0.2)	202.2	18.9	32.2	20.9
October	85.1 (8.6)	14.3 (0.2)	4	16.7	39.4	17.8
November	105.6 (10.7)	9.3 (0.4)	116.8	10.2	95.4	9.9
December	75.5 (7.7)	5.3 (0.3)	93	8.7	20.2	7.6
January	54.4 (6.4)	4.5 (0.3)	72.2	6.2	36.4	6.5

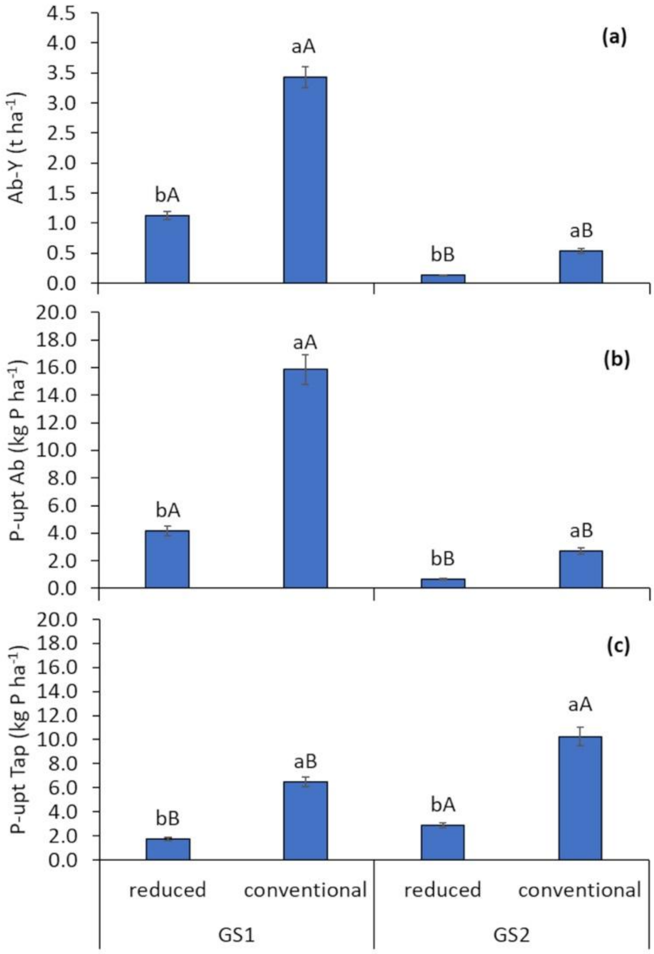

Figure 2 Effect of growing season (GS1 and GS2) and tillage (reduced and conventional) interaction on aboveground biomass yield (Ab-y; **a**), phosphorus uptake by aboveground biomass (P-upt Ab; **b**), and phosphorus uptake by taproot (P-upt Tap; **c**). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. Lowercase letters indicate significant differences between tillage management, while uppercase letter indicate significant differences between growing seasons.

The P-upt Ab was 3.8 times higher in GS1 and 4 times higher in GS2 under conventional tillage compared to reduced tillage (Figure 2b). Similarly, P-upt Tap under reduced tillage was significantly lower by approximately 72% in both GS1 and GS2 compared to conventional tillage (Figure 2c). The lower P uptake in roots under reduced tillage likely reflects differences in soil mixing and residue decomposition between tillage systems. Conventional tillage enhances residue incorporation and organic matter mineralization, increasing nutrient availability in the rooting zone (Palm et al., 2014; Sithole et al., 2019). This process, driven by greater soil disturbance, promotes microbial activity and accelerates nutrient release, including phosphorus (Six et al., 2000; VandenBygaart et al., 2002). In contrast, reduced tillage, with limited soil turnover, may slow residue decomposition and nutrient release, contributing to the observed differences in P-upt Tap.

Across all conditions, Ab-Y, Tap-Y, P-upt Ab, and P-upt Tap were significantly higher under conventional tillage than under reduced tillage. These findings highlight the importance of soil turnover and nutrient release in driving TR performance, particularly in low-fertility soils. Reduced tillage may require additional amendments, such as nitrogen fertilization or phosphorus-enriched organic materials, to support comparable biomass production and nutrient uptake (Yagioka et al., 2014; Pavinato et al., 2017).

Overall, P-upt Ab in GS1 was about 6 times higher than in GS2 with both tillage systems. The decrease in P uptake during GS2 was likely due to lower rainfall during the growing season (Supplementary Table 2). Pavinato et al. (2017) reported that limited rainfall can reduce P solubilization and uptake due to the restricted soil moisture, which is also critical for root activity and nutrient diffusion. Under reduced tillage, P-upt Tap in GS2 was about 62.9% higher than in GS1, while under conventional tillage, the increase was around 57.7%. These findings suggest that higher rainfall in GS1 may have facilitated greater P translocation from roots to shoots as observed by Hansen et al. (2021), who noted a similar root P uptake of 7.9 kg P ha^{-1} under comparable conditions.

The seasonal patterns revealed that Ab-Y and P-upt Ab were higher in GS1 than in GS2, while Tap-Y and P-upt Tap showed the opposite trend, being higher in GS2. These differences are likely related to rainfall distribution. The higher rainfall in GS1 may have supported greater aboveground growth and nutrient translocation to shoots, while the lower rainfall in GS2 may have prompted increased resource allocation to root development as a strategy to enhance water and nutrient acquisition. Similar root-shoot allocation dynamics in response to environmental stress have been documented in *Raphanus sativus* (Hansen et al., 2021). White and Weil (2011) also observed that forage radish exhibited increased root allocation under moisture-limited conditions, a strategy to enhance water and nutrient acquisition. Such dynamics are consistent with Reza et al. (2020), who documented adaptive root-shoot allocation in Brassicaceae cover crops in response to environmental stress, highlighting their capacity to adjust biomass distribution based on prevailing conditions.

Table 3 Analysis of variance of the studied soil parameters (variation; **Δ**) affected by the considered factors and their interactions. The F value column indicates the F values of the ANOVA results, while the sig columns reports the different significant levels (* = 0.05, ** = 0.01, *** = 0.001, ns= not significant).

Variability sources	**Df**	**ΔSOC (g C kg^{-1})**		**ΔPOM-C (mg C kg^{-1})**		**ΔPOX-C (mg C kg^{-1})**		**ΔMBC (mg C kg^{-1})**		**ΔOlsen P (mg P kg^{-1})**		**ΔMBP (mg P kg^{-1})**		**ΔN tot (mg N kg^{-1})**		**ΔMBN (mg N kg^{-1})**	
		F	**sig**	**F**	**sig**	**F**	**sig**	**F**	**sig**	**F**	**sig**	**F**	**sig**	**F**	**sig**	**F**	**sig**
GS	1	17.34	***	1.83	ns	0.33	ns	10	**	0.58	ns	10.13	**	2.05	ns	2.86	ns
Till	1	73.43	***	26.68	***	0.49	ns	26.1	***	30.76	***	11.84	***	21.7	***	9.51	**
GC	1	109.91	***	76.75	***	1.73	ns	1932	***	133.18	***	1457.25	***	26.38	***	529.95	***
SDe	2	4.48	ns	0.14	ns	1.31	ns	19.3	***	4.03	ns	4.35	ns	3.81	ns	1	ns
GS:Till	1	0.02	ns	0.76	ns	0.19	ns	0.05	ns	0.39	ns	0.5	ns	12.21	***	2.16	ns
GS:GC	1	2.34	ns	0.73	ns	2.43	ns	26.6	***	0.65	ns	0.68	ns	0.39	ns	0.26	ns
Till:GC	1	43.19	***	27.3	***	0.01	ns	5.1	*	49.32	***	2.79	ns	21.98	***	0.65	ns
GS: SDe	2	1.13	ns	0.07	ns	0.05	ns	8.94	***	0.59	ns	0.32	ns	5.03	**	0.11	ns
Till: SDe	2	2.04	ns	3.38	*	1.58	ns	5.49	**	0.59	ns	2.44	ns	0.35	ns	2.33	ns
GC: SDe	2	1.17	ns	0.1	ns	4.05	*	9.08	***	1.19	ns	6.87	**	1.4	ns	0.06	ns
GS:Till:GC	1	2.02	ns	0.8	ns	0.11	ns	0.16	ns	1.92	ns	2.19	ns	3	ns	0.09	ns
GS:Till: SDe	2	0.12	ns	1.97	ns	1.01	ns	0.15	ns	1.86	ns	0.06	ns	0.11	ns	0.95	ns
GS:GC: SDe	2	0.35	ns	0.04	ns	0.18	ns	0.52	ns	0.16	ns	3.41	*	0.45	ns	1.14	ns
Till:GC: SDe	2	6.22	**	0.24	ns	0.01	ns	0.37	ns	0.81	ns	1.97	ns	1.2	ns	3.68	*
GS:Till:GC:SDe	2	0.14	ns	0.42	ns	0.06	ns	0.56	ns	0.32	ns	0.37	ns	0.95	ns	1.15	ns
Residuals	408																

GS: Growing Seasons (GS1 vs GS2); Till: Tillage system (reduced vs conventional); GC: Ground Cover (cover crop vs no cover crop); SDe: Soil Depth (0-5 cm, 5-10 cm, and 10-20 cm)

ΔSOC: variation of soil organic carbon; ΔPOM-C: variation of particulate organic carbon; ΔPOX-C: variation of permanganate oxidable carbon; ΔMBC: variation of microbial biomass carbon; ΔOlsen-P: variation of Olsen phosphorus; ΔMBP: variation of microbial biomass phosphorus; ΔN tot: variation of total nitrogen; ΔMBN: variation of microbial biomass nitrogen

Table 4 Effect of the main factors on the variation of the considered soil parameters. Values are means with standard errors in parentheses. Different letters indicate significant differences within each factor according to Tukey's post-hoc test

Variability sources	ΔSOC ($g\ C\ kg^{-1}$)	ΔPOM-C ($mg\ C\ kg^{-1}$)	ΔPOX-C ($mg\ C\ kg^{-1}$)	ΔMBC ($mg\ C\ kg^{-1}$)	ΔOlsen P ($mg\ P\ kg^{-1}$)	ΔMBP ($mg\ P\ kg^{-1}$)	ΔN tot ($mg\ N\ kg^{-1}$)	ΔMBN ($mg\ N\ kg^{-1}$)
GS								
GS1	0.17 (0.02) b	22 (3.5)	-0.87 (0.65)	44.89 (3.76) a	0.12 (0.02)	1.02 (0.09) a	6.3 (2.7)	3.28 (0.3)
GS2	0.24 (0.03) a	15.7 (4.6)	-0.39 (0.64)	41.31 (3.46) b	0.1 (0.02)	0.85 (0.09) b	1.8 (2.6)	2.77 (0.39)
Till								
Reduced	0.1 (0.02) b	6.9 (3.3) b	-0.34 (0.61)	40.66 (3.03) b	0.05 (0.01) b	0.84 (0.08) b	-3.2 (2.4) b	2.56 (0.32) b
Conventional	0.31 (0.03) a	30.9 (4) a	-0.92 (0.56)	45.53 (3.55) a	0.17 (0.02) a	1.03 (0.08) a	11.3 (2.3) a	3.48 (0.31) a
GC								
CC	0.4 (0.03) a	39.3 (4.1) a	-0.08 (0.64)	86.79 (1.74) a	0.23 (0.02) a	1.96 (0.05) a	12 (2.8) a	6.47 (0.25) a
nCC	0 (0.02) b	-1.5 (3.4) b	-1.18 (0.65)	-0.59 (1.34) b	-0.01 (0.02) b	-0.09 (0.04) b	-3.90(2.3) b	-0.42 (0.21) b
SDe								
0_5_cm	0.24 (0.04)	20.5 (5.4)	-1.53 (0.76)	41.28 (3.87)	0.15 (0.02)	0.92 (0.08)	10.1 (3.50)	3.32 (0.42)
5_10_cm	0.15 (0.03)	17.5 (4.8)	0.09 (0.8)	38.78 (3.91)	0.1 (0.02)	0.84 (0.08)	1.30 (3.00)	2.85 (0.45)
10_20_cm	0.21 (0.03)	18.7 (4.9)	-0.45 (0.8)	49.24 (5.3)	0.08 (0.02)	1.04 (0.09)	0.70 (3.2)	2.9 (0.4)

GS: Growing Season; Till: Tillage system; GC: Ground Cover; SDe: Soil Depth: CC, cover crop; nCC, non-cover crop;

ΔSOC: variation of soil organic carbon; ΔPOM-C: variation of particulate organic carbon; ΔPOX-C: variation of permanganate oxidable carbon; ΔMBC: variation of microbial biomass carbon; ΔOlsen-P: variation of olsen phosphorus; ΔMBP: variation of microbial biomass phosphorus; ΔN tot: variation of total nitrogen; ΔMBN: variation of microbial biomass nitrogen

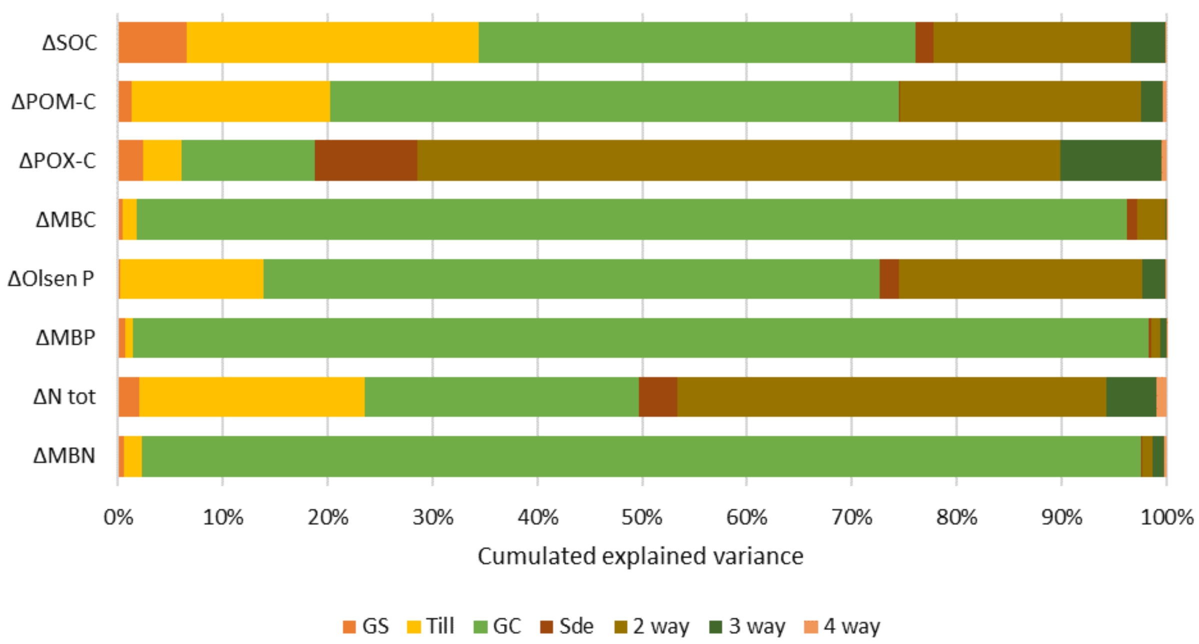

Figure S1 Cumulated explained variance for the considered parameters influenced by the main factors and their interactions (2-way, 3-way, and 4-way interactions)

5.4.2. Impact of tillage, cover crop, and soil depth on soil organic carbon dynamics

The variation in soil organic carbon (ΔSOC) was significantly affected by the triple interaction of tillage (Till), ground cover (GC), and soil depth (SDe) (TillxGCxSDe) (Table 3). Across all tillage treatments, cover crop (CC) consistently showed significantly higher ΔSOC compared to non-cover crop (nCC) (Figure 3). These findings confirm the effectiveness of TR in increasing SOC across soil depths in low-fertility soils. The results agree with Wang et al. (2024), who observed a similar increase in SOC at the 0-20 cm depth when TR was grown without manure application compared to fallow. Conversely, Wang et al. (2017) reported minimal differences in SOC between radish and fallow in the 0-30 cm layer, highlighting the variability in SOC response based on specific management practices and environmental conditions. Across GC, ΔSOC values under conventional tillage were significantly higher than those under reduced tillage by 75.9%, 73.5%, and 60.0% for the 0-5 cm, 5-10 cm, and 10-20 cm soil layers, respectively. The initial reduction in SOC under reduced tillage can likely be attributed to lower biomass input, as documented by Tang et al. (2021) in the transition to conservation tillage. Reduced tillage often results in slower decomposition of residues and lower carbon inputs, delaying SOC accumulation in the short term. In contrast, conventional tillage promotes residue incorporation and organic matter decomposition, creating favorable conditions for tillage radish growth and carbon input, particularly in surface layers (Blanco-Canqui and Lal, 2009).

A significant variation in SOC between soil depths was observed, but only under conventional tillage. In this system, the 0-5 cm layer exhibited SOC levels 61% and 39% higher than the 5-10 cm and 10-20 cm layers, respectively. Additionally, the GC treatment significantly influenced ΔSOC, as confirmed by its contribution to the explained variance (reported in Figure S1). (Figure S1). The observed differences can be attributed to favorable conditions for tillage radish growth and carbon input under conventional tillage, which promotes residue incorporation and organic matter accumulation in surface layers. These results were in agreement with Hu et al. (1997), who reported greater SOC accumulation in the upper soil layers in systems with winter cover crops compared to fallow management. Blanco-Canqui and Lal (2009) reported that conventional tillage enhances soil loosening and residue incorporation, supporting root establishment and nutrient access. Similarly, Kahlon et al. (2013) found that conventional tillage increases soil organic carbon in surface layers through enhanced residue incorporation. In contrast, reduced tillage often results in slower residue decomposition and delayed nutrient availability (Giller et al., 2009). The ΔSOC was significantly higher in GS2 compared to GS1 (Table 4), likely due to lower rainfall and soil moisture in GS2, which may have reduced SOC mineralization.

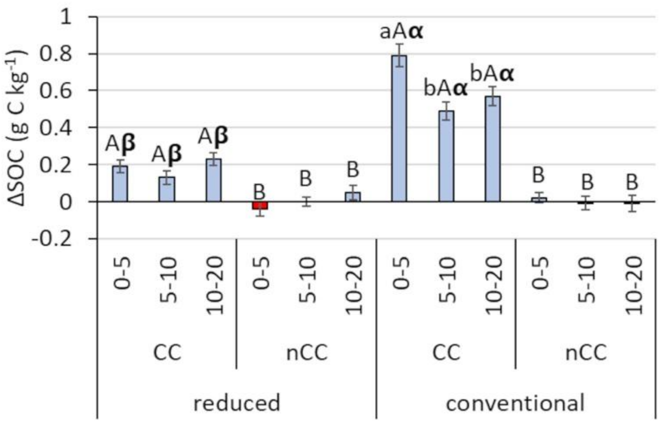

Figure 3 Effect of the interaction between tillage (reduced vs conventional), ground cover (cover crop –CC– vs non-cover crop –nCC) and soil depth (0-5 cm, 5-10 cm, and 10-20 cm) on the variation of soil organic carbon (ΔSOC). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. Lowercase letters represent significant differences between soil depths according to the post-hoc Tukey's test. Uppercase letters specify significant differences between the ground cover management Greeks letters indicate significant difference between tillage management

5.4.3. Effects of tillage, soil depth, and cover crops on labile carbon dynamics

The interactions Till x SDe and Till x GC significantly influenced changes in particulate organic matter carbon (ΔPOM-C) (Table 3). Under reduced tillage, ΔPOM-C in the 0-5 cm and 10-20 cm layers was 98.3% and 75.3% lower, respectively, compared to conventional tillage (Figure 4a). This increase in POM-C content in the top layer under conventional tillage agrees with Engedal et al. (2023), who observed similar results for TR. Additionally, the increase in POM-C in the 10-20 cm layer under conventional tillage is likely due to the incorporation of crop residues into deeper layers, which enhances the decomposition of residues and contributes to POM-C formation (Ahmad et al. 2023; Cotrufo and Lavallee, 2022). Cover crops (CCs) further increased ΔPOM-C, with values 16.5 and 65.2 mg C kg^{-1} higher in reduced and conventional tillage, respectively, relative to bare soil. This difference is consistent with the higher biomass yield of TR, particularly under conventional tillage, where TR growth was enhanced. Kuneski et al. (2023) similarly reported increased POM-C stocks in the 0–30 cm soil layer with oilseed radish treatments. However, under reduced tillage with CCs, the ΔPOM-C was 76.2% lower than under conventional tillage (Figure 4b), underscoring the effect of biomass yield in driving POM-C accumulation. Overall, ΔPOM-C was significantly higher with conventional tillage than with reduced tillage, and in plots with cover crops compared to those without CC (Table 4). The combined effects of GC and Till explained 73% of the total explained variance of ΔPOM-C (Figure S1), emphasizing the importance of tillage and CC management in regulating SOC dynamics.

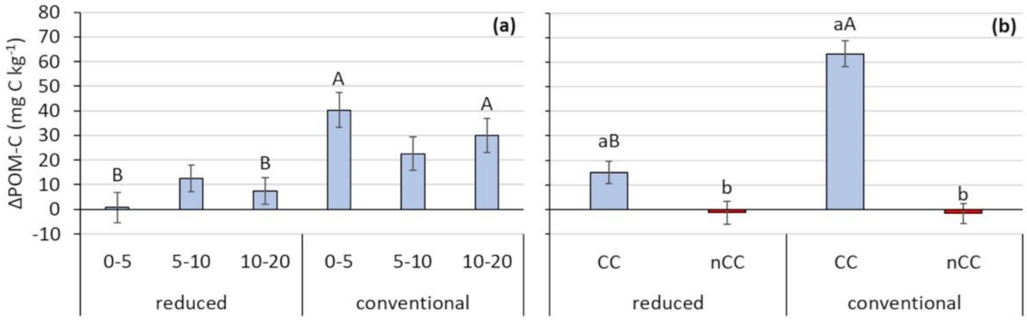

Figure 4 Effect of the tillage x soil depth (**a**), and tillage x ground cover (**b**) interactions on the variation of soil particulate-organic matter carbon (ΔPOM-C). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. In the Till x SDe interaction (a), uppercase letters specify significant differences between tillage management (reduced vs conventional). In the Till x GC (b), lowercase letters indicate significant differences between ground cover management (cover crop –CC– vs non-cover crop –nCC). Uppercase letters specify significant differences between tillage management.

Regarding the variation in permanganate oxidable carbon (ΔPOX-C), it was significantly affected by the interaction GC x SDe (Table 3). Without cover crop (nCC), ΔPOX-C in the intermediate layer was nearly three times higher than in the 0-5 cm layer, with no significant difference with the 10-20 cm layer (Figure 5). In contrast, with cover crop (CC), ΔPOX-C in the 10-20 cm layer was significantly higher by 3.2 mg POX-C kg^{-1} than without cover crop. These findings are consistent with Wang et al. (2017), who reported an even greater effect of TR on POX-C at 15–30 cm depth compared to the surface layer, suggesting that the residue decomposition of TR contributes significantly to labile carbon pools at deeper depths. The negative values in the upper soil layers could be explained by the poor development of TR under reduced tillage, which likely resulted in decreased labile C inputs into the soil due to limited biomass production and residue turnover. This explanation is consistent with Blanco-Canqui and Lal (2009), who noted that reduced tillage systems often lead to slower decomposition rates and lower labile carbon contributions, particularly in the surface layers. Moreover, the limited effect of main factors on ΔPOX-C (Table 4), highlights that soil depth is a crucial factor determining how ground cover management promotes changes in labile C dynamics. Two-way interactions collectively explained about 61.4% of the total explained variance in ΔPOX-C, while GC, SDe, and three-way interactions together accounted for an additional 32.1% (Figure S1). This highlights the complexity of factors influencing labile carbon pools and underscores the significance of interactive effects between cover crops and soil depth in promoting changes in labile carbon. These results emphasize the potential of TR as a cover crop to enhance labile carbon fractions, particularly in deeper soil layers where residue inputs are more effectively integrated. However, the limited impact observed in the upper layers under reduced tillage suggests that further research is needed to optimize the performance of TR and other cover crops under conservation tillage systems, especially in low-fertility soils.

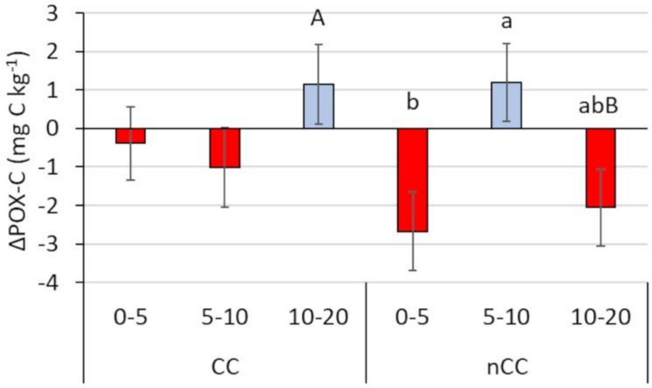

Figure 5 Effect of the interaction ground cover (cover crop –CC– vs non-cover crop –nCC) and soil depth (0-5 cm, 5-10 cm, and 10-20 cm) on the variation of soil permanganate-oxidable carbon (ΔPOX-C). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. Lowercase letters represent significant differences between soil depths according to the post-hoc Tukey's test. Uppercase letters specify significant differences between ground cover management.

The interactions GC × SDe, Till × CC, and GS × GC significantly affected the variation in microbial biomass carbon (ΔMBC) (Table 3). Across all soil depths, ΔMBC was consistently higher with cover crop than without cover crop (Figure 6a). In particular, with cover crop, ΔMBC in the 10-20 cm layer was significantly higher by 31% and 25% compared to the 5-10 cm and 0-5 cm layers, respectively, suggesting enhanced decomposition of TR residues and microbial activity in the deeper soil layers. This effect, where MBC increase was higher in deeper layers, contrasts with typical findings that report higher microbial activity in surface layers compared to deeper layers due to greater organic inputs and root activity (Chen et al. 2016; Kaiser and Heinemeyer, 1993). However, the higher ΔMBC in deeper layers suggests that TR residues were effectively decomposed and incorporated into the soil profile. Under both reduced and conventional tillage, ΔMBC was significantly higher with cover crop, by 80.9 and 93.9 mg C kg^{-1}, respectively, than without cover crop (Figure 6b). This highlights the role of cover crops in enhancing microbial biomass by providing organic matter as a substrate for microbial activity. However, with cover crop, ΔMBC under reduced tillage was about 12.3% lower compared to conventional tillage, likely due to the greater biomass yield of TR with this soil management. These findings align with Bhagat et al. (2024), who found a significant increase in MBC in the upper 15 cm with radish compared to bare soil. Seasonal effects were also evident, as ΔMBC with cover crop was significantly higher in GS1 by 91 mg C kg^{-1} than without cover crop, meanwhile in GS2, it was higher by 83.7 mg C kg^{-1} (Figure 6c). The consistent ΔMBC increase with cover crop across seasons, slightly higher in GS1 than in GS2, highlights TR residues' reliable contribution to microbial biomass. Overall, ΔMBC was significantly higher in GS2 than in GS1, with cover crop than without it, and with conventional tillage than with reduced tillage (Table 4). These results underscore the importance of GC in driving microbial biomass dynamics, as most of the variance in ΔMBC (94.5%) was explained by GC (Figure S1). TR shows potential to enhance microbial biomass, particularly under conventional tillage with effective residue incorporation. The lower ΔMBC under reduced tillage highlights the need for strategies to improve TR residue decomposition in conservation systems.

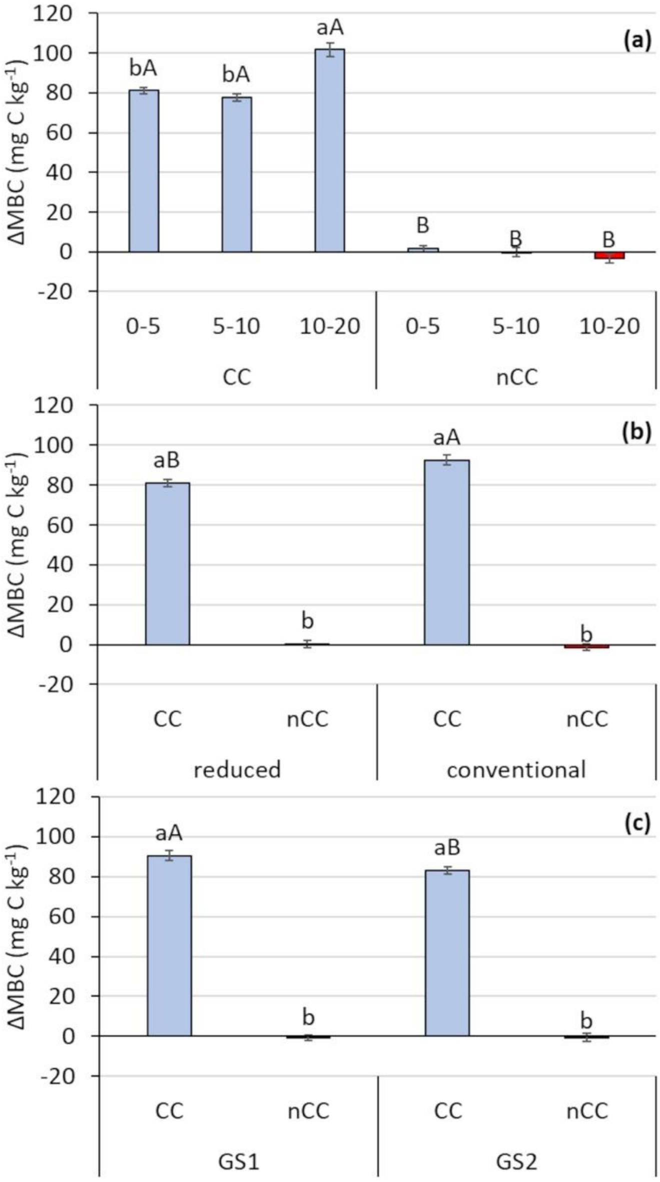

Figure 6 Effect of the ground cover x soil depth (**a**; GC x SDe), tillage x ground cover (**b;** Till x GC), and growing season x ground cover (**c**; GS x GC) interactions on the variation of soil microbial carbon (ΔMBC). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. In GC x SDe (a), lowercase letters specify significant differences between SDe (0-5 cm, 5-10 cm, and 10-20 cm), while uppercase letters indicate significant differences between GC (cover crop –CC– vs non-cover crop –nCC). In Till x GC (b), lowercase letters indicate significant differences between ground cover management, while uppercase letters specify significant differences between tillage management (reduced vs conventional). In GS x GC (c), lowercase letters specify significant differences between GC, while uppercase letters indicate significant differences between growing seasons (GS; GS1 vs GS2).

5.4.4. Effects of tillage, soil depth, and cover crops on the soil phosphorus availability

Among the double interactions, the effect of Till × GC on the difference in Olsen-P (ΔOls-P) was particularly significant (Table 3). In total, the double interactions accounted for approximately 23% of the total explained variance (Figure S1). Under conventional tillage, ΔOls-P with cover crop and without cover crop was 0.39 mg P kg^{-1}, while under reduced tillage, ΔOls-P with cover crop was significantly higher by 0.09 mg P kg^{-1} than without cover crop (Figure 7). Although these differences in ΔOls-P between treatments were statistically significant, the actual magnitude of the difference was minimal and likely not impactful for fertilizer management decisions. The robustness of our findings is supported by the large number of field and analytical replications, which may have contributed to detecting these small differences. Thus, these differences indicate a trend in increasing available P to crops more than a practical impact after two growing seasons. These results are consistent with findings by Soltangheisi et al. (2018), who reported a significant increase in soil P test (SPT) in forage radish compared to fallow under similar conditions. In our study, with cover crop, ΔOls-P under conventional tillage was three times higher than under reduced tillage, likely due to the greater biomass yield of tillage radish enhancing phosphorus availability. This suggests that, beyond mobilizing P from the soil, the turnover of P accumulated in cover crop residues contributes significantly to P availability by recycling P absorbed by cover crop into the soil. The results are consistent with White and Weil (2011), who suggested that intensive tillage systems with higher biomass input increases Olsen P compared to fallow systems. Overall, ΔOls-P increased with conventional tillage compared to reduced tillage, and with cover crop relative to fallow (Table 4). In this regard, the GC factor alone explained about 59% of the total explained variance in ΔOls-P, highlighting the strong influence of cover crop management on phosphorus availability (Figure S1).

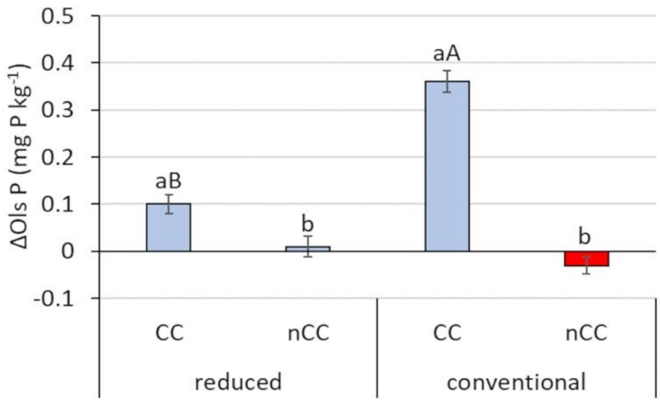

Figure 7 Effect of the interaction between tillage (reduced vs conventional) and ground cover (cover crop –CC– vs non-cover crop –nCC) on the variation of soil Olsen Phosphorus (ΔOls P). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. Lowercase letters represent significant differences between ground cover . Uppercase letters specify significant differences between tillage management.

The GS × GC × SDe interaction significantly affected the variation in microbial biomass phosphorus (ΔMBP) (Table 3). In both GSs, ΔMBP consistently increased with cover crop across all soil depth (Figure 8). With cover crop during GS1, ΔMBP at 10-20 cm depth was 18.2% higher than in the 0-5 cm layer, in this latter layer ΔMBP being 14.7% higher than in the 5-10 cm layer. Comparing both GSs, significant differences in ΔMBP with cover crop were detected only in the 0-5 cm and 10–20 cm layers, where values were 17% and 19.4% higher, respectively, in GS1 than in GS2. These findings contrast with studies, such as Joshi and Garkoti (2023), which reported a decline in MBP with increasing soil depth across various vegetation types. This discrepancy could be attributed to microbial activity degrading TR residues in the deeper soil layers. The observed increase in MBP may result from enhanced microbial biomass driven by the organic carbon supply from TR residues, which actively contribute to the soil phosphorus cycle (Wang et al. 2021). Overall, ΔMBP was significantly higher in GS1 compared with GS2, under conventional tillage compared with reduced tillage, and with cover crop compared with bare soil (Table 4). These results suggest that ploughing enhances microbial biomass and P turnover, contrasting with the expectations of higher SOC and microbial biomass under reduced tillage (Carter, 1992). This can be explained by the accelerated decomposition and mineralization of residues with intensive tillage, that favors soil aeration and microbial activity. As observed for ΔMBC, the majority of variance in ΔMBP was explained by the GC factor (Figure S1). The greater TR development during GS1, likely due to higher rainfall than in GS2, provided an important source of organic carbon for the soil, stimulating microbial activity. In contrast, the higher temperatures during GS2 may have delayed the winterkill of the cover crop, decreasing decomposition rate as observed by Hashemi et al. (2013).

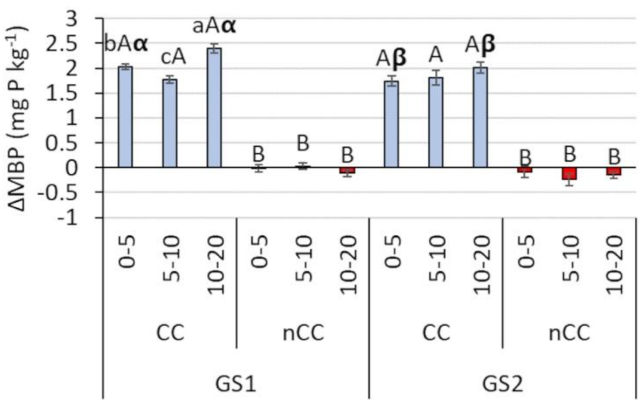

Figure 8 Effect of the interaction between growing season (GS1 vs GS2), ground cover (cover crop –CC– vs non-cover crop –nCC), and soil depth (0-5 cm, 5-10 cm, and 10-20 cm) on the variation of soil Microbial Biomass Phosphorus (ΔMBP). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. Lowercase letters represent significant differences between soil depths according to the post-hoc Tukey's test. Uppercase letters specify significant differences between the ground cover management.. Greeks letters indicate significant difference between growing season.

5.4.5. Effect of the analyzed factors on the soil nitrogen dynamics

The interactions GS × SDe, Till × GC, and GS X Till exerted a significant effect on the variation in total nitrogen (ΔNtot) (Table 3). Two-way interactions explained about 41% of the total explained variance (Figure S1). In GS2, ΔNtot in the 0-5 cm layer was significantly higher by 21 and 17.4 mg N kg^{-1} compared to the 5-10 cm and 10-20 cm layers, respectively (Figure 9a). In the 10-20 cm layer, ΔNtot in GS1 exceeded that in GS2 by 14.2 mg N kg^{-1}, likely reflecting better TR performance in GS1. Under conventional tillage, ΔNtot with cover crop was significantly increased by 30.5 mg N kg^{-1} compared to bare soil (Figure 9b). On the other hand, with cover crop, ΔNtot under conventional tillage was 29 mg N kg^{-1} higher than in reduced tillage, emphasizing the role of residue incorporation on soil nitrogen dynamics. The increase in total N with TR was likely ascribed to the recycling soil N by absorbing it and reducing losses from the soil (Fraser et al. 2013). This agrees with Rushing et al. (2023), who detected a similar increase in total N with radish cover crops compared to fallow. In GS2, ΔNtot under conventional tillage was 25 mg N kg^{-1} higher than under reduced tillage (Figure 9c). Conversely, under reduced tillage, ΔNtot was 15.2 mg N kg^{-1} higher in GS1 than in GS2, possibly reflecting the impact of TR's poor performance during GS2. Overall, ΔNtot was significantly higher under conventional tillage compared to reduced tillage and with cover crop compared to bare soil (Table 4). This is likely ascribed to the enhanced performance of TR under conventional tillage.

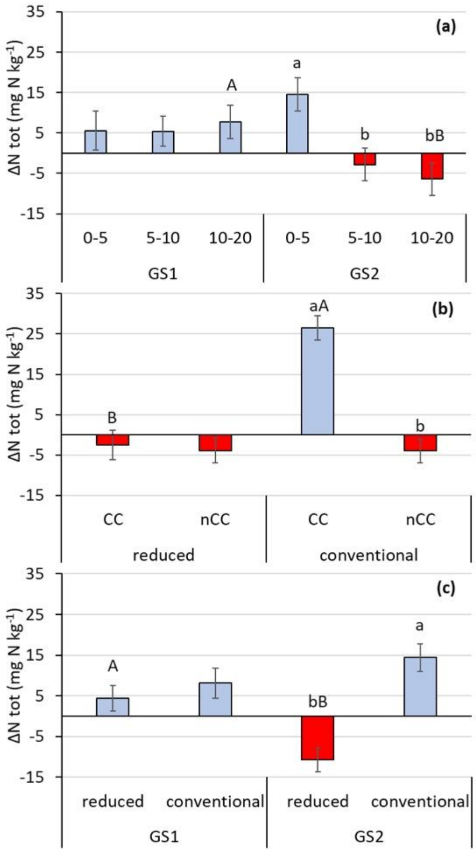

Figure 9 Effect of the growing season x soil depth (**a**; GS x SDe), tillage x ground cover (**b;** Till x GC), and growing season x tillage (**c**; GS x Till) interactions on the variation of soil Total Nitrogen (ΔNtot). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. In GC X SDe (a), lowercase letters indicate significant differences between SDe (0-5 cm, 5-10 cm, and 10-20 cm), while uppercase letters specify significant differences between GS (GS1 vs GS2). In Till X GC (b), lowercase letters indicate significant differences between GC (cover crop –CC– vs non-cover crop –nCC), while uppercase letters specify significant differences between Till (reduced vs conventional). In GS X Till, lowercase letters indicate differences between Till, while uppercase letters specify significant differences between GS

The interaction Till × GC × SDe significantly affected the variation in microbial biomass nitrogen (ΔMBN). Without cover crop, MBN increase varied with SDe in both reduced and conventional tillage (Figure 10). Under reduced tillage without cover crop, the ΔMBN in the top 5 cm was 2 mg N

kg^{-1} higher than in 5-10 cm layer, but not significantly different from the 10-20 cm layer. Under conventional tillage without cover crop, the ΔMBN in the 5-10 cm layer was 1.43 mg N kg^{-1} higher than in 10-20 cm layer, with no significant differences between the top layer and the others. A significant difference between tillage methods was detected in the 5-10 cm layer without cover crop, where ΔMBN under conventional tillage was approximately three times higher than under reduced tillage. This pattern is consistent with the MBP results, suggesting that ploughing boosts microbial biomass, through enhanced soil aeration and organic matter decomposition. Under reduced tillage, this initial increase may be less pronounced, potentially leading to a more stable and gradual decline over time. The impact of the tillage factor on MBN was less pronounced than in previous studies (Silva et al. 2010), possibly due to the relatively short time since the transition to reduced tillage. On the other hand, the positive effect of TR on MBN was evident even in this short-term study, in agreement with Balota et al. (2014) who observed this effect with radish in long-term studies. The accumulation of nitrogen in both residues and microbial biomass contributes to nitrogen retention and reduces losses in the soil system. Overall, the ΔMBN values were increased by conventional tillage relative to reduced tillage, and with cover crop compared to bare soil (Table 4). The total explained variance in ΔMBN was largely attributed to GC (95%), as previously observed in ΔMBC and ΔMBP (Figure S1), thus highlighting the relevance of the cover crop relevance on these variables.

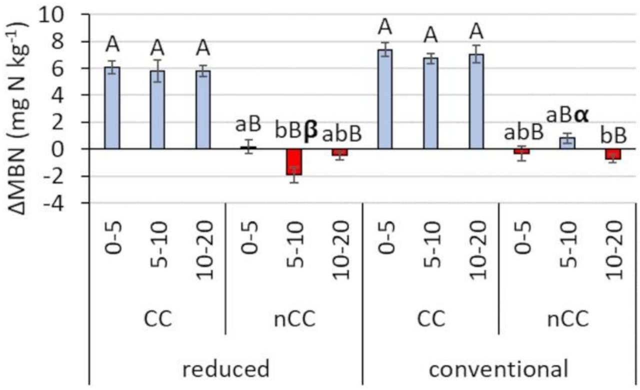

Fig. 10 Effect of the interaction between tillage (reduced vs conventional), ground cover (cover crop –CC– vs non-cover crop –nCC) and soil depth (0-5 cm, 5-10 cm, and 10-20 cm) on the variation of soil Microbial Biomass Nitrogen (ΔMBN). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. Lowercase letters represent significant differences between soil depths according to the post-hoc Tukey's test. Uppercase letters specify significant differences between the ground cover management. Greeks letters indicate significant difference between tillage.

5.4.6. Effect of the analyzed factors on the soil physical properties

Table 5 Analysis of variance of the physical soil parameters, i.e. bulk density (BD) and degree of compactness (DC). The F column indicates the F values of the ANOVA results, while the sig columns report the different significant levels (* = 0.05, ** = 0.01, *** = 0.001, ns= not significant).

Variability sources	DF	BD ($t\ m^{-3}$)		DC (%)	
		F	sig	F	sig
Samp	1	227.95	***	490.33	***
Till	1	388.52	***	282.65	***
GC	1	5.57	ns	0.03	ns
Sde	3	1583.39	***	1156.61	***
Samp:Till	1	234.08	***	49.54	***
Samp:GC	1	0.68	ns	0.29	ns
Till:GC	1	23.84	***	0.75	ns
Samp:Sde	3	23.19	***	12.43	***

Till:Sde	3	63.71	***	43.19	***
GC:Sde	3	0.27	ns	0.03	ns
Samp:Till:GC	1	0.19	ns	0.01	ns
Samp:Till:Sde	3	24.79	***	3.29	*
Samp:GC:Sde	3	0.47	ns	0.04	ns
Till:GC:Sde	3	0.16	ns	0.42	ns
Samp:Till:GC:Sde	3	0.06	ns	0.46	ns
Residuals	1120				

Table 6 Effect of the main factors sampling [Samp; PRE vs POST], tillage [Till; reduced vs conventional], ground cover [GC; CC vs nCC], and soil depth [SDe; 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm] on the variation of the bulk density (BD) and degree of compactness (DC). Values are means with standard errors in parentheses.

Variability sources	**Levels**	**BD ($t\ m^{-3}$)**	**DC (%)**
Samp	PRE	1.41 (0.05)	0.82 (0.03)
	POST	1.42 (0.04)	0.84 (0.03)
Till	Reduced	1.43 (0.05)	0.83 (0.03)
	Conventional	1.41 (0.04)	0.82 (0.03)
GC	CC	1.42 (0.05)	0.83 (0.03)
	nCC	1.42 (0.04)	0.83 (0.03)
SDe	0-5 cm	1.36 (0.02)	0.79 (0.01)
	5-10 cm	1.40 (0.03)	0.83 (0.02)
	10-20 cm	1.44 (0.04)	0.84 (0.02)
	20-30 cm	1.46 (0.02)	0.85 (0.01)

The interaction between sampling time (Samp), tillage system (Till), and soil depth (SDe) significantly affected bulk density (BD) (Table 5). Regardless of tillage and sampling time, BD increased with soil depth (Figure 11). Before TR sowing (PRE), BD in the 0–5 cm layer was 1.5% higher under conventional tillage than reduced tillage, while after TR cultivation (POST), BD was 1.1% higher under reduced tillage. In deeper layers (10–20 cm and 20–30 cm), BD was 1.4% and 1.1% higher under reduced tillage before TR sowing, with these differences increasing to 3.5% and 1.6% after TR cultivation. Under conventional tillage, BD increased by 0.6% in the 0–5 cm layer from PRE to POST, while in the 5–10 cm layer, BD was 0.4% lower at POST than PRE. BD in cover crop (CC) plots under reduced tillage was 0.7% higher than in bare soil plots, while with CC, BD under conventional tillage was 2.1% lower than under reduced tillage (Figure 12).

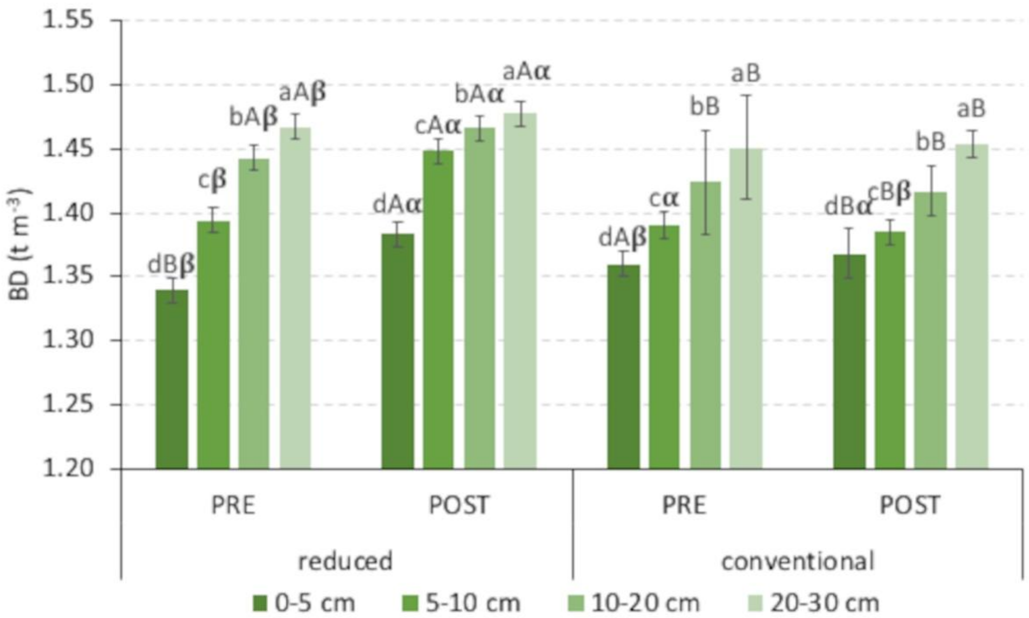

Figure 11 Effect of the interaction between tillage (reduced vs conventional), sampling (PRE vs POST), and soil depth (SDe; 0-5 cm, 5-10 cm, 10-20 cm, and 20-30 cm) on soil Bulk Density (BD). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. Lowercase letters represent significant differences between soil depths, within Samp and Till, according to the post-hoc Tukey's test. Uppercase and Greeks letters specify significant differences between Till, within Samp and SDe, and between Samp, within Till and SDe, respectively.

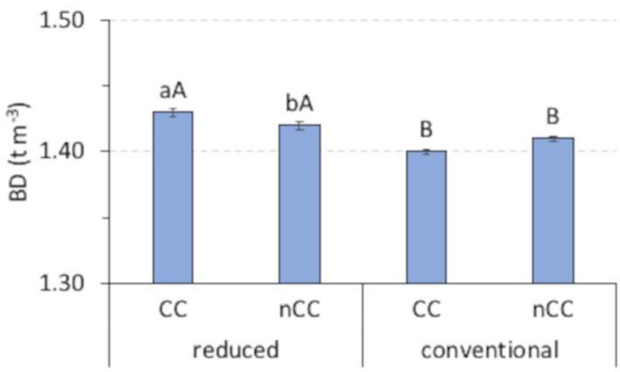

Figure 12 Effect of the interaction between tillage (reduced vs conventional) and ground cover (CC vs nCC) on soil Bulk Density (BD). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. Lowercase letters represent significant differences between soil ground cover. Uppercase letters specify significant differences between tillage management.

The Samp × Till × SDe interaction also significantly affected degree of compactness (DC) (Table 5). DC increased with soil depth (Figure 13). Before TR sowing, DC was significantly higher under conventional tillage across all depths, while after TR cultivation, DC was significantly higher under reduced tillage. POST values were consistently higher than PRE, except at 10–20 cm under conventional tillage.

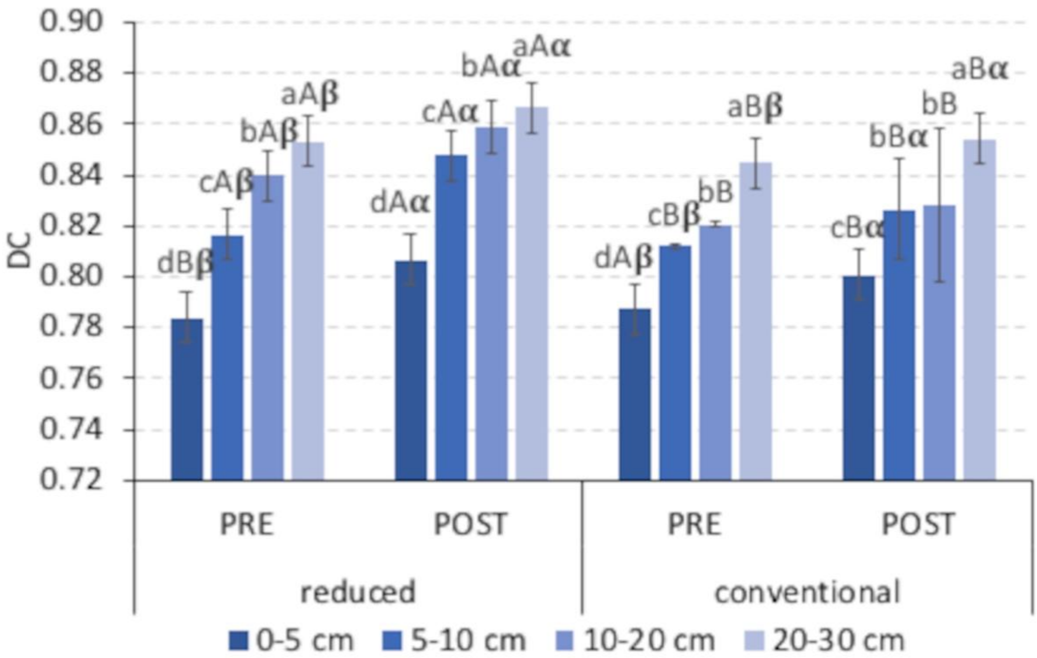

Figure 13 Effect of the interaction between tillage (reduced vs conventional), sampling (PRE vs POST), and soil depth (SDe; 0-5 cm, 5-10 cm, 10-20 cm, and 20-30 cm) on soil Degree of Compactness (DC). Error bars indicate standard errors. Letters indicate significant differences within each simple effect analysis. Lowercase letters represent significant differences between SDe according to the post-hoc Tukey's test. Uppercase letters specify significant differences between tillage management. Greeks letters indicate significant difference between sampling.

Soil compactness is influenced by intrinsic properties such as texture and organic matter content but also varies based on crop type and climatic conditions (Suzuki et al., 2006; Reichert et al., 2009; Keller and Håkansson, 2010). However, absolute bulk density (BD) alone is not a reliable indicator of soil compaction's impact on crop yield, as optimal and critical BD thresholds depend significantly on soil-specific properties (Håkansson, 1990; Reichert et al., 2009; Keller and Håkansson, 2010). A BD value indicating compaction in one soil type may correspond to a loose structure in another (Håkansson, 1990), underscoring the need for a more adaptable evaluation approach when assessing soil physical conditions for plant growth.

To address this variability, Håkansson (1990) proposed the degree of compactness (DC) as a more consistent reference, establishing a correlation between crop yield and DC values across different soil types. In 100 field experiments with spring barley (Hordeum vulgare L.), he found that maximum crop yield was consistently achieved at a DC of 0.87, irrespective of the mineral soil type. However, Håkansson and Lipiec (2000) refined this framework by demonstrating that optimal DC values vary between crop species. While 0.87 is optimal for barley, wheat, and sugar beet, other crops require progressively looser soil conditions, including legumes and oats (~0.85), oilseeds and field beans (~0.84), and tuber crops (~0.82) due to their larger root systems and higher sensitivity to compaction.

Although BD and DC differences in this study were statistically significant, their small magnitude suggests limited agronomic relevance. These variations likely reflect short-term structural changes induced by TR biomass accumulation and root activity, with long-term effects requiring further research. Nonetheless, soil compaction levels remained within acceptable limits for most cash crops, regardless of cover crop (CC) presence or tillage system (reduced or conventional). Consequently, TR's benefits in mitigating soil compaction may be more relevant for crops with lower DCopt values, particularly in reduced tillage systems, where compaction tends to persist over time.

5.5. Conclusions

Tillage radish provides tangible short-term benefits to soil, particularly when its growth and biomass incorporation are optimized through appropriate tillage practices. Soil loosening optimize its benefits on soil properties. Accordingly, total organic and labile C were consistently higher in treatments with greater tillage radish biomass input. This study confirmed the ability of tillage radish to increase soil available phosphorus in low-P soils. This increase was related to root biomass development, with the highest values detected under conventional tillage. Tillage radish also increased total N and microbial biomass indicators (microbial biomass C, P, and N), which were higher under conventional tillage than under reduced tillage, highlighting the enhanced nutrient cycling potential with greater biomass incorporation. The bulk density was consistently higher under reduced tillage, particularly in deeper soil layers, while tillage radish effectively reduced bulk density under conventional tillage, where its growth was more vigorous. Similarly, the degree of compactness followed a comparable trend, with values under reduced tillage approaching thresholds that could limit root development. The study was carried out on a single soil during only two seasons. This limits the generalizability of these findings to other conditions. Further research is needed to evaluate the effect of tillage radish on soil properties across diverse soil types, tillage systems, and cropping conditions to fully optimize its agricultural and environmental benefits.

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6. Third paper

Tillage radish as cover crop improves soil health indicators depending on pedoclimatic conditions

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6.1. Abstract

Replacing fallow periods with cover crops (CCs) is a key conservation agriculture measure to enhance soil health, yet its effectiveness depends on pedoclimatic conditions. However, the effects of CCs, such as tillage radish (TR), on soil health indicators under different environmental and management conditions remain insufficiently unexplored. This study assessed the effects of TR on soil health indicators related to carbon (C), nitrogen (N), and phosphorous (P) cycling in two locations (Signa –SN– and Cesa –CS–) under conservation agriculture with different pedoclimatic conditions. Biomass accumulation and P uptake by TR were significantly higher at SN than CS, likely due to more favorable soil and climatic conditions. The higher biomass at SN resulted in threefold higher accumulation of soil organic carbon (SOC) in the upper 5 cm compared to CS. TR increased SOC, labile organic C, soil P test, and microbial biomass C, N, and P, though most effects varied by location. Soil P test increased with TR biomass, confirming its role in P mobilization. At SN, TR improved nitrogen retention in the soil by about 34 mg N kg^{-1} compared to fallow. These findings highlight TR's positive effects on some soil health indicators related to relevant ecosystem services, particularly C storage, microbial biomass, P mobilization and recycling, and N balance, with site-specific variations depending on pedoclimatic conditions. Future studies should explore the long-term impacts of TR on soil fertility and nutrient cycling across diverse agroecosystems involving different management practices and pedoclimatic conditions for solid practical recommendations.

Keywords: conservation agriculture, regenerative agriculture, ecosystem services, nutrient cycling, reduced tillage

6.2. Introduction

The application of Conservative Agriculture (CA) practices has recently been included in the Common Agriculture Policy (CAP) 2023-2027 as a condition for direct payments for environmentally friendly practices (EU Commission, 2023). These practices now account for 30 % of all eco-schemes at EU level. In particular, soil conservation practices together with the sowing of cover crops (CCs) have been included in the suggested practices for applying CA. One of the main reasons for prompting these practices is the declining levels of soil organic matter (SOM), which pose a significant threat to EU soils (De Rosa et al. 2024). Nevertheless, at a global scale, the area under CA accounts for around 15% of the total cropland area, while, it represents less than 6% of the total EU cropland area (Kassam et al. 2022).One of the main barriers for the adoption of CA at the EU level is the knowledge gap that hinders the widespread of CA practices (Kassam et al. 2019). In particular, the short-term uncertainties regarding the agronomic outcomes of CA could have played an essential role in the poor adoption of CA among farmers (Achankeng and Cornelis 2023; Pisante et al. 2015). Therefore, testing these practices under different pedoclimatic conditions and cropping systems is pivotal for their wider adoption.

The sowing of CCs facilitates the adoption of cropping systems such as double cropping, intercropping, and relay cropping, optimizing the use of available growing periods. Their effects on soil heath and ecosystem serviceshave been demonstrated by various authors (Adil et al. 2022; Burgess et al. 2022; Garba et al. 2024). The sowing of a legume crop as a CC has been shown to be a useful strategy for providing ecosystem services and enriching the soil with nitrogen without further external inputs (Domnariu et al. 2024; Lamichhane and Alletto 2022). Therefore, legumes CCs have been already included in the suggested practices for applying CA (EU Commission, 2023). Conversely, the benefits of non-legume CCs are not yet fully understood (Wong et al. 2024). Some non-legume CCs grow faster than legume CCs, making them ideal for situations requiring rapid soil cover for erosion control (Dabney et al. 2001). Among non-legume CCs, brassicas, such as radishes, offer additional benefits. For instance, they are used as green manure, can release phosphatase enzymes into the soil which hydrolyze organic phosphorus (P) compounds, increasing P availability to plants (Solangi et al. 2019). In fact, some studies have shown that tillage radish (TR) can effectively increase Soil P Test (SPT) levels in both conventional and no tillage systems (White and Weil 2011). However, the impacts of TR on SPT levels need further exploration, particularly in soils with low P availability. So far, few studies have examined changes in P fractions following the sowing of TR in systems transitioning to CA.

Biomass accumulation of CCs has already been identified as a critical factor for providing ecosystem services (Cafaro La Menza and Carciochi 2023). A recent review highlighted that winter CCs are able to increase both stable and labile organic C in the upper 5 cm depth, though these benefits are generally observed only under irrigation conditions (Blanco-Canqui et al. 2024). On the other hand, the same authors reported that at least 2 t ha^{-1} of biomass could be required to increase significantly organic C. In the early years of transitioning from conventional to reduced tillage, CCs often struggle to accumulate significant dry matter (Merkle et al. 2024). This reduced establishment success could be attributed to the difficulty CCs face in rooting and growing in soils not loosened by conventional tillage practices. Soil texture has been identified as an important factor influencing the adoption of CA (Van den Putte et al., 2010). The same authors noted that root and tuber crops are generally less affected in the transition to the conservation agriculture. This suggests that TR, as a CC, could be highly effective across different soil textures, particularly due to its robust root system and adaptability to reduced tillage systems.

Non-legume CCs also play an essential role in nitrogen (N) capture, absorbing soil inorganic nitrogen and storing it in their biomass as organic nitrogen (Thapa et al. 2018). By absorbing N, CCs mitigate the risk of N loss by leaching, runoff, and gaseous emissions (Blanco-Canqui 2023). However, the capacity of non-legume CCs to scavenge N depends on their dry matter accumulation. Cottney et al. (2022) demonstrated that TR, when sown early, was particularly effective in accumulating N in its tissues. Another recent study found that TR residues release approximately 43% of total accumulated nitrogen for uptake by the next cash crop (Greub and Roberts 2023). Most of the N stored in TR residues is released through mineralization (Ketterings et al. 2015). In the short term, this process can increase the microbial biomass nitrogen (MBN) pool, especially when compared to fields left fallow (Xing et al. 2022). However, the effects of TR residues on MBN pools and overall N cycling remain underexplored.

In the present study, we investigated the potential of TR to replace fallow periods. The study was conducted over two consecutive growing seasons in two environments with different soil types and climatic conditions, different cropping systems, and but with the same reduced tillage practices, which had been implemented for less than five years - a relatively short period to observe the benefit of this tillage system. Since CCs are known to deliver benefits once they accumulate at least 2 t ha^{-1} of dry matter (Blanco Canqui et al. 2024), the study aimed to evaluate the capacity of TR to establish under these different soil and climate conditions across growing seasons and how it affects soil health indicators related to C, N, and P cycling and storage in soil. The goal was to determine whether TR as a CC could provide short-term soil health benefits compared to leaving the fields fallow.

6.3. Materials and methods

6.3.1. Site description

The experiment took place under rainfed conditions at two locations (Fig. 1): the Tereto Living Lab (CS) in Marciano della Chiana, Arezzo, Italy (43°19’5’’ N, 11°47’43’’ E, 246 m asl) and Signa (SN) in Florence, Italy (43°47’49’’ N, 11°5’1’’ E, 34 m asl). It was conducted over two consecutive growing seasons, 2022-2023 and 2023-2024, referred to as GS1 and GS2, respectively. The soil in CS is a typical Vertic Calciustepts according to Soil Taxonomy (Soil Survey Staff, 2014) with a clay loam texture, while the soil in SN is a Fluvaquentic Dystroxerepts (Soil Survey Staff, 2014) with a silt loam texture. The specific properties of the two soils are schematized in Table 1.

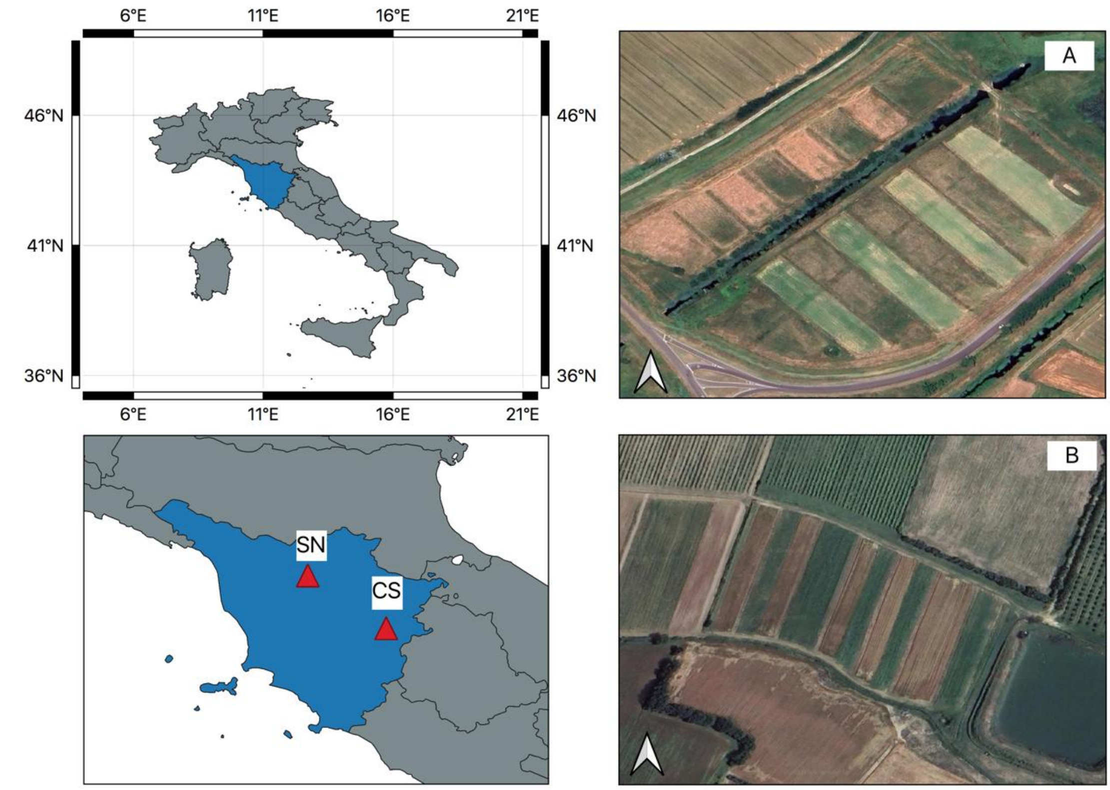

Figure 1 Maps of the experimental fields: Panel A represents the Signa location (SN), while Panel B represents the Cesa location (CS)

Both locations have a hot-summer Mediterranean climate (Csa), experiencing dry and hot summers and wet autumn. According to the long-term data (1991-2020), the average temperature from August to January is higher in SN (14.5 °C) than in CS (12.5 °C). In both GSs and locations, the average temperature was 2 °C higher than the long-term data. Long-term rainfall data from August to January indicate an average of 431 mm and 463 mm, for CS and SN, respectively, representing about 58% and 62% of the total annual rainfall in CS and SN, respectively. During GS1, CS experienced an average of 556 mm of rainfall from August to January, but this dropped significantly to 252 mm during GS2. SN recorded higher rainfall, with 590 mm in GS1 and 387 mm in GS2.

Table 1 Initial soil characterization of Tereto Living Lab (CS) and Signa (SN) for the upper 15 cm soil layer. Mean ± standard deviation values are reported.

Soil property	**CS**	**SN**
	Value ± SD	**Value ± SD**
Sand (%)	32.82 ± 1.81	21.54 ± 2.70
Silt (%)	32.24 ± 0.94	53.81 ± 2.94
Clay (%)	34.94 ± 2.05	24.65 ± 3.53
pH_w (1:5)	8.20 ± 0.03	7.10 ± 0.02
EC (dS m^{-1})	0.20 ± 0.01	0.14 ± 0.01
CEC ($cmol_c$ kg^{-1})	28.97 ± 1.44	16.80 ± 0.34
$CaCO_3$ (%)	5.62 ± 0.17	0.01 ± 0.01
Soil Organic Carbon (%)	1.00 ± 0.55	0.90 ± 0.36
Total Nitrogen (%)	0.10 ± 0.06	0.07 ± 0.03
Olsen P (mg P kg^{-1})¥	7.90 ± 0 .56	9.47 ± 0.95

pH_w= pH in 1:5 soil water/extract; CEC=cation exchange capacity; EC= electrical conductivity in 1:2.5 soil water/extract

6.3.2. History before the sowing of tillage radish in both locations

The field at CS is located in one of Tuscany's main areas for cultivation of herbaceous crop. The field has a long history of conventional cropping. Briefly, the system alternates winter and spring crops, primarily sunflower (*Helianthus annuus* L.) and durum wheat (*Triticum turgidum* subsp. durum), since 2015. Fields are left fallow from July to March, creating an opportunity for a winter cover crop like TR. Before 2021, conventional tillage involved mouldboard ploughing to a depth of 30 cm, followed by disc harrowing at 10 cm. After 2021, reduced tillage practices were adopted, involving chisel ploughing to 30 cm depth, followed by disc harrowing at 10 cm.

The field at SN was managed as a stable lawn system untill 2018, a common practice in the lowlands around Florence, Italy (Pescatore et al. 2023). In 2018, the soil was moldboard ploughed to a depth of 30 cm for the first and only time, followed by disk harrowing at 10 cm. Since then, the soil has been chisel ploughed at 30 cm and disk harrowed at 10 cm in late summer in preparation for the spring sowing of crimson clover (*Trifolium incarnatum* L.) and white clover (*Trifolium repens* L.).

6.3.3. Experimental design and management

At CS, the experimental layout was a split-plot design, with ground cover (GC) management treatments (cover crop vs. no cover crop) assigned as the main factor. The field was divided into three blocks, each containing 12 plots (six replicates per treatment per block), resulting in a total of 18 replicates per treatment. The plot size was 15 m × 15 m (225 m^2). At SN, the experimental design was a completely randomized block design, with three blocks and three replicates per block, resulting in nine replicates per treatment. The GC management treatments (cover crop vs. no cover crop) were randomly assigned to the plots within each block. The plot size was 25 m × 25 m (625 m^2).

In both locations and GSs, TR was sown between the 2nd and 3rd weeks of September at a seeding rate of 18 kg ha^{-1}. The seeds were broadcasted and, after sowing, a spring tine harrow was used in all plots to kill emerged weeds and improve seed-to-soil contact in cover crop plots. No fertilizers or pesticides were applied in either GS and locations. After sowing, no additional management practices were required, except for the termination of TR. At CS, TR was terminated in February with glyphosate at 2 L ha^{-1}, whereas at SN, the chemical termination was unnecessary as TR was naturally terminated by winter frost.

6.3.4. Biomass and soil sampling

In both locations, CC biomass was sampled prior to termination during the second half of January in both GSs. Two 50 cm X 50 cm quadrats were randomly harvested in each plot to represent the plot's biomass. TR biomass was divided into aboveground and belowground components to assess dry matter accumulation. The determination of P uptake in different plant parts was carried out by ashing 0.4 g of dried plant tissue samples in a muffle furnace at 550°C for 5 hours. The resulting ash was dissolved in 40 mL of 0.3 M HCl and filtered using Whatman no. 42 filter paper. Phosphorus concentration in the filtrate was then analyzed using the vanadomolybdophosphoric acid method as described by Kuo (1996).

Soil samples were collected at both locations and during both GSs, prior to TR sowing and again during its late growth stages (around mid- to late January at both locations in each GS). Soil samples were taken from depths (Sde) of 0-5 cm, 5-10 cm, and 10-20 cm layers. Before the analysis, soil samples were air dried and sieved to 2 mm. Soil organic carbon and total N were analyzed using a CHN analyzer (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA) after grinding soil with a planetary ball mill (Giovannini et al. 1975; Buckee et al. 1994). The measurement of particulate organic carbon (POM-C) and permanganate oxidable carbon (POX-C) were performed following

Cambardella and Elliott (1992) and Weil et al. (2003), respectively. Microbial carbon (MBC), phosphorus (MBP), and nitrogen (MBN) were determined by the fumigation-extraction method (Vance et al. 1987; Brookes et al. 1985; Vance et al. 1985). Briefly, MBC and MBN were extracted from fumigated and non-fumigated soil samples using 0.5 M K_2SO_4, whereas MBP was extracted using 0.5 M $NaHCO_3$. The resulting extracts were analyzed using a CHN analyzer for MBC and MBN (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA) and colorimetrically for MBP (Lambda 20 Spectrophotometer, Perkin Elmer Corporation, Waltham, MA, USA). Correction factors for MBC, MBP, and MBN were 0.38, 0.40, and 0.54, respectively.

6.3.5. Statistical analysis

The R environment (R Core Team, 2021) was used for all analyses. Normal distribution was tested using the Shapiro-Wilk test, and the homogeneity of variances was tested by Levene's test ('car' package; Fox and Weisberg 2019). When assumptions were not met, data were transformed using the Box-Cox transformation ('MASS' package; Venables and Ripley 2002).

For dry matter accumulation and P uptake, the effects of location (CS vs. SN) and GS (GS1 vs GS2) were analyzed using ANOVA with the model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \epsilon$$

Where β_0 is the general average, $\beta_1 X_1$and $\beta_2 X_2$ represent the effect of location and GS, respectively, and $\beta_3 X_1 X_2$ is their interaction (location x GS).

Soil properties were analyzed by calculating the differences (Δ) between measurements taken after and before the cultivation of TR at both locations. . Since the main aim of this paper is to assess the influence of TR on soil properties under different conservation systems, the following model, which evaluates the effects of multiple factors ($X_1$to X_4) and their interaction on changes in soil properties (Y_{soil}), was used:

$$Y_{soil} = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i<j} \beta_{ij} X_i X_j + \sum_{i<j<k} \beta_{ijk} X_i X_j X_k + \beta_{1234} X_1 X_2 X_3 X_4 + \epsilon$$

Where X_3 and X_4 represent the GC and Sde, resepectively.

To assess changes in soil parameters, data collected at the end of each TR growing season were subtracted from the initial values measured just before the sowing of TR. The assumptions of normal distribution for soil data were tested using the Shapiro-Wilk test, while homogeneity of variances was assessed using Levene's tests. When the assumptions were not met, data were transformed using the Box-Cox transformation. Then, the transformed data were subjected to ANOVA and Tukey's test for assessing any potential differences between growing season, location, soil depth and treatments, as well as their interactions. The ANOVA results were also used to calculate the explained variance of each factor as a proportion of the total explained variance. This was determined from the sum of squares for each factor, considering main effects and interactions while excluding residual variance. This approach quantifies the relative contribution of each factor to the dependent variable.

6.4. Results

6.4.1. Effect of location and growing season on the biomass and phosphorus accumulation of tillage radish

The parameters aboveground yield (Ab-Y), taproot yield (Tap-Y), and phosphorus uptake by aboveground biomass (P-upt Ab) were significantly influenced by the interaction between growing season and location (GS X Loc, Table 2). In GS1, the Ab-Y value recorded at SN was nearly three times higher than at CS (Fig. 2a). During GS2, the Ab-Y at SN was approximately six times higher than at CS. At CS, the Ab-Y measured in GS2 showed an 88.5% reduction compared to GS1, while the Ab-Y measured in GS2 at SN was reduced by about 83% compared to GS1.

In both GSs, the Tap-Y recorded at SN was about four times higher than that at CS (Fig. 2b). In GS1, the P-upt Ab at SN was approximately four times higher than at CS (Fig. 2c). In GS2, the P-upt Ab value at SN was nearly fivefold higher than at CS. At CS, the P-upt Ab measured in GS2 was reduced by 84.3% compared to GS1, while at SN, the P-upt Ab in GS2 was 79.4% lower than in GS1.

In general, all measured parameters (Ab-Y, Tap-Y, and P-upt Ab) were significantly higher at SN than at CS. Both Ab-Y and P-upt Ab were significantly higher in GS1 than in GS2, whereas Tap-Y and phosphorus uptake by taproot (P-upt Tap) were significantly increased in GS2 compared to GS1.

Table 2 Mean values (± standard error) and ANOVA results for tillage radish aboveground biomass yield (Ab-Y), taproot biomass yield (Tap-Y), aboveground phosphorus uptake (P-upt Ab), and taproot phosphorus uptake (P-upt Tap) across two locations (Loc; Cesa and Signa) and two growing seasons (GS; GS1 and GS2). The 'sig' columns report the different significant levels (* = 0.05, *** = 0.001, ns= not significant)

Variability sources	df	Ab-Y		Tap-Y		P-upt Ab		P-upt Tap	
		t ha^{-1}	Sig	t ha^{-1}	Sig	kg P ha^{-1}	Sig	kg P ha^{-1}	Sig
Loc	1		***		***		***		***
CS		0.63 ± 0.09 b		0.51 ± 0.03 b		2.40 ± 0.34 b		2.31 ± 0.15 b	
SN		2.14 ± 0.39 a		2.02 ± 0.09 a		9.59 ± 1.75 a		9.05 ± 0.44 a	
GS	1		***		ns		***		***
GS1		1.92 ± 0.26 a		0.90 ± 0.13		8.07 ± 1.25 a		3.85 ± 0.60 b	
GS2		0.34 ± 0.06 b		1.12 ± 0.16		1.52 ± 0.26 b		5.27 ± 0.69 a	
GS x Loc	1		***		*		***		ns

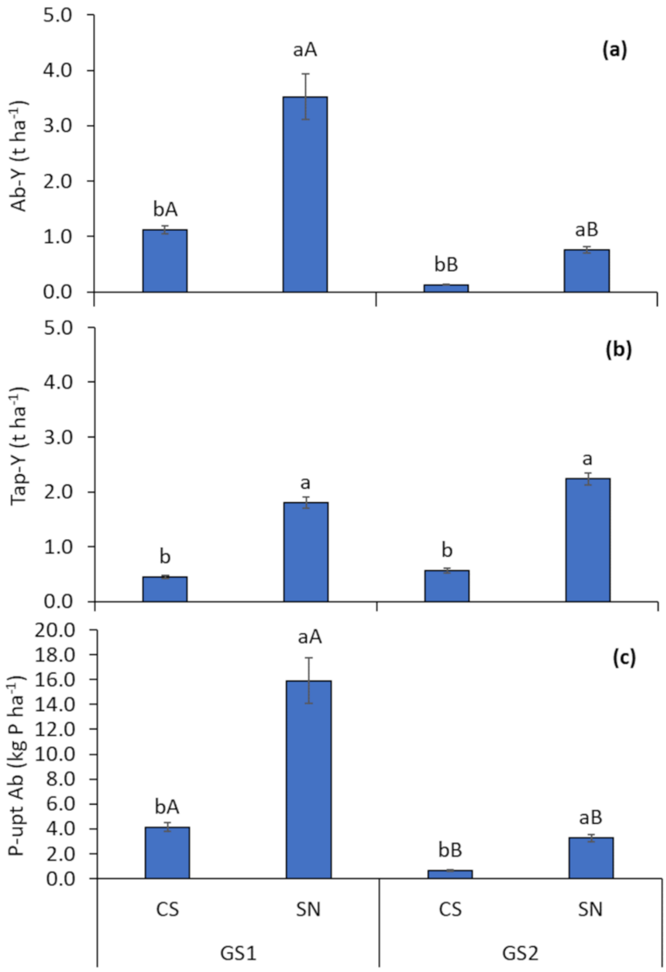

Figure 2 (a) Effect of growing season and location interaction (GS X Loc) on aboveground biomass yield (Ab-y), **(b)** taproot yield (Tap-Y), and **(c)** phosphorus uptake by aboveground biomass (P-upt Ab). Error bars indicate standard error. Lowercase letters indicate significant differences between tillage management, while uppercase letters indicate significant differences between growing seasons (GS) according to the Tukey HSD test.

Table 3 ANOVA for various soil properties, showing the effects of the main factors (Growing Season, Location, Ground Cover, and Soil Depth) and their interactions. F column indicates the F-statistic values, while the Sig columns reports the different significant levels (* = 0.05, ** = 0.01, *** = 0.001, ns= not significant).

Variability sources	Df	ΔSOC ($g C kg^{-1}$)		ΔPOM-C ($g C kg^{-1}$)		ΔPOX-C ($mg C kg^{-1}$)		ΔMBC ($mg C kg^{-1}$)		ΔOlsen P ($mg P kg^{-1}$)		ΔMBP ($mg P kg^{-1}$)		ΔN tot ($g N kg^{-1}$)		ΔMBN ($mg N kg^{-1}$)	
		F	Sig	F	Sig	F	Sig	F	Sig	F	Sig	F	Sig	F	Sig	F	Sig
GS	1	0.19	ns	1.88	*	0.01	ns	0.81	ns	0.49	ns	0.8	ns	10.4	**	7.01	**
Loc	1	15.42	***	6.92	**	5.42	*	28.25	***	8.4	**	4.19	*	0.19	ns	2.26	ns
GC	1	155.77	***	16.48	***	9.67	**	1776.99	***	20.82	***	741.7	***	19.51	***	261.8	***
Sde	2	7.71	***	0.41	ns	0.40	ns	13.86	***	4.77	**	6.8	ns	0.22	ns	3.16	ns
GS x Loc	1	7.68	**	0	ns	0.07	ns	6.34	*	0.38	ns	6.09	*	3.13	ns	0.01	ns
GS x GC	1	0.04	ns	0.28	ns	0.12	ns	0.62	ns	1.95	ns	2.68	ns	1.1	ns	0.66	ns
Loc x GC	1	6.59	*	5.06	*	7.57	**	17.42	***	0.62	ns	0.01	ns	23.91	***	4.96	*
GS x Sde	2	1.91	ns	0.11	ns	0.76	ns	4.43	ns	1.95	ns	0.01	ns	1.78	ns	0.93	ns
Loc x Sde	2	26.48	***	0.38	ns	2.15	ns	25.42	***	0.21	ns	3.61	*	1.09	ns	1.14	ns
GC x Sde	2	8.94	***	1.69	ns	0.20	ns	5.02	**	1.99	ns	1.04	ns	0.27	ns	1.65	ns
GS x Loc x GC	1	8.18	**	0.38	ns	1.42	ns	4.27	*	0.38	ns	6.79	**	0	ns	0.62	ns
GS x Loc x Sde	2	0.23	ns	0.36	ns	0.04	ns	2.28	ns	0.82	ns	0.14	ns	0.68	ns	1.68	ns
GS x GC x Sde	2	0.25	ns	0.02	ns	0.06	ns	1.82	ns	0.03	ns	2.21	ns	0.32	ns	0.26	ns
Loc x GC x Sde	2	6.17	**	1.31	ns	2.42	ns	29.02	***	4.49	*	5.72	**	0.35	ns	5.08	**
GS x Loc x GC x Sde	2	1.71	ns	0.59	ns	0.38	ns	1.44	ns	0.09	ns	2.25	ns	0.83	ns	2.47	ns
Residuals	336																

GS = growing seasons (GS1 and GS2); Loc = location (Cesa and Signa); GC = ground cover (cover crop and no cover crop); SDe = soil depth (0-5 cm, 5-10 cm, and 10-20 cm)

ΔSOC= variation of soil organic carbon; ΔPOM-C= variation of particulate organic carbon; ΔPOX-C= variation of permanaganate oxidable carbon; ΔMBC= variation of microbial biomass carbon; ΔOlsen P= variation of Olsen phosphorus; ΔMBP= variation of microbial biomass phosphorus; ΔN tot= variation of total nitrogen ; ΔMBN= variation of microbial biomass nitrogen

Table 4 Effect of the main factors on the variations of soil chemical properties. Values represent the differences (Δ) between measurements taken after and before radish cultivation. Reported values are means ± standard error. Different letters indicate significant differences ($p<0.05$) within the main factors according to the Tukey HSD test.

Variability sources	ΔSOC (g C kg^{-1})	ΔPOM-C (mg C kg^{-1})	ΔPOX-C (mg C kg^{-1})	ΔMBC (mg C kg^{-1})	ΔOlsen P (mg P kg^{-1})	ΔMBP (mg P kg^{-1})	ΔN tot (mg N kg^{-1})	ΔMBN (mg N kg-1)
GS								
GS1	0.12 ± 0.02	19.9 ± 6.30 a	0.85 ± 0.84	37.61 ± 3.09	0.09 ± 0.02	0.93 ± 0.09	2.70 ± 2.60 a	3.28 ± 0.31 a
GS2	0.13 ± 0.02	9.7 ± 4.20 b	0.72 ± 0.87	36.02 ± 3.30	0.07 ± 0.02	0.87 ± 0.09	-7.90 ± 2.20 b	2.30 ± 0.38 b
Loc								
CS	0.10 ± 0.02 b	6.90 ± 3.30 b	-0.34 ± 0.61 b	40.66 ± 3.03 a	0.05 ± 0.01 b	0.84 ± 0.08 b	-3.20 ± 2.40	2.56 ± 0.32
SN	0.17 ± 0.01 a	26.8 ± 6.60 a	2.48 ± 0.97 a	31.04 ± 2.72 b	0.12 ± 0.02 a	0.99 ± 0.09 a	-1.70 ± 2.00	3.13 ± 0.32
GC								
CC	0.23 ± 0.02 a	29.9 ± 5.30 a	2.63 ± 0.98 a	74.22 ± 1.85 a	0.14 ± 0.02 a	1.88 ± 0.06 a	4.60 ± 2.50 a	5.77 ± 0.28 a
nCC	0.01 ± 0.01 b	-0.20 ± 5.20 b	-1.06 ± 0.69 b	-0.59 ± 1.23 b	0.03 ± 0.02 b	-0.08 ± 0.05 b	-9.8 ± 2.30 b	-0.2 ± 0.26 b
SDe								
0-5 cm	0.17 ± 0.02 a	12.20 ± 5.80	0.25 ± 1.07	43.35 ± 4.09 a	0.13 ± 0.02 a	1.07 ± 0.12	-1.10 ± 2.90	3.44 ± 0.43
5-10 cm	0.09 ± 0.02 b	19.50 ± 6.90	1.51 ± 0.91	34.38 ± 3.65 b	0.08 ± 0.02 ab	0.75 ± 0.11	-3.50 ± 2.90	2.37 ± 0.48
10-20 cm	0.11 ± 0.02 b	12.70 ± 7.00	0.60 ± 1.15	32.71 ± 3.97 b	0.04 ± 0.02 b	0.89 ± 0.10	-3.20 ± 3.20	2.56 ± 0.36

GS =Growing Seasons (GS1 vs GS2); Loc = location (Cesa vs Signa); GC = ground cover (cover crop vs no cover crop); SDe = soil depth (0-5 cm, 5-10 cm, and 10-20 cm)

ΔSOC= variation of soil organic carbon; ΔPOM-C= variation of particulate organic carbon; ΔPOX-C= variation of permanaganate oxidable carbon; ΔMBC= variation of microbial biomass carbon; ΔOlsen P= variation of Olsen phosphorus; ΔMBP= variation of microbial biomass phosphorus; ΔN tot= variation of total nitrogen ; ΔMBN= variation of microbial biomass nitrogen

6.4.2. Effect of the analyzed factors in reduced tillage on the soil carbon dynamics

Across all conditions, ΔSOC values were consistently higher in cover crop (CC) treatments than in no cover crop (nCC) treatments (Fig. 3a). The interaction of Loc × GC × Sde had a significant effect on ΔSOC (Table 3). At SN, ΔSOC in the 0-5 cm layer was 2.3 and 4.5 times greater than in the 5-10 cm and 10-20 cm layers, respectively. Within CC, ΔSOC in the 0-5 cm layer at SN was approximately three times higher than that at CS, while in the 5-10 cm layer, SN exhibited a significantly higher ΔSOC by 45.8% compared to CS. Within nCC, a notable increase of 0.12 g C kg^{-1} in ΔSOC was observed in the 0-5 cm layer at SN compared to CS.

The three-way interaction of GS × Loc × GC significantly influenced ΔSOC (Fig. 3b). The impact of CC on SOC was most pronounced in treatments with the highest TR biomass accumulation, particularly during GS1 at SN. Here, ΔSOC in CC was 12 times higher than in nCC. During GS1 under CC, ΔSOC measured at SN was 2.6 times greater than that at CS. Additionally, within CC at SN, ΔSOC during GS1 was significantly higher by 44% compared to GS2. Conversely, within CC at CS, ΔSOC in GS2 was 64.3% higher than in GS1. Overall, the majority of ΔSOC variance (63%) was explained by GC factor (Fig. S1).

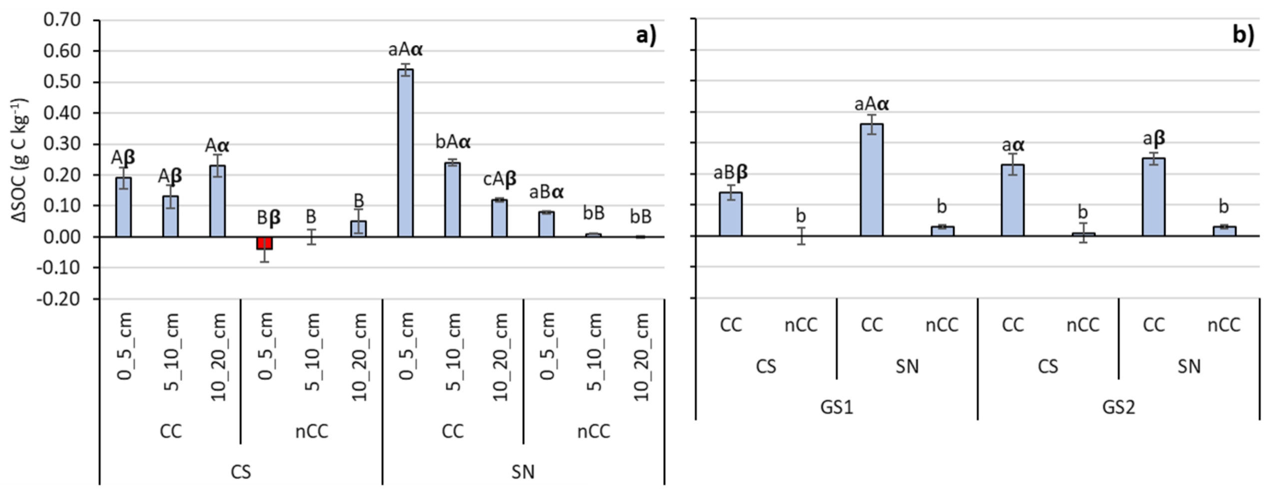

Figure 3 (a) Effect of the interaction between location (CS vs SN), ground cover (CC vs nCC), and soil depth (0-5, 5-10, 10-20 cm) on the variation of soil Organic Carbon (ΔSOC). **(b)** Effect of the interaction between growing season (GS1 vs GS2), location (CS vs SN), and ground cover (CC vs nCC) on the variation of soil Organic Carbon (ΔSOC). Error bars indicate standard error. Lowercase, uppercase and Greek letters indicate significant differences between levels of the same factor according to the Tukey HSD test. Lowercase letters represent significant differences between soil depth, while uppercase letters specify significant differences between ground cover. Greek letters indicate significant differences between locations.

A significant effect of Loc x GC interaction on ΔPOM-C was observed (Table 3). At CS, a significant increase of 16.5 mg C kg^{-1} in ΔPOM-C was detected in CC compared to nCC (Fig. 4). Additionally, within CC, the ΔPOM-C measured at SN was 3.5 times compared to that measured at CS. Ground cover alone explained 46% of the total variance in ΔPOM-C, while two-way interactions accounted for 21%, and location contributed 19% to the total variance (Fig. S1). Overall, a significant increase in ΔPOM-C was observed in GS1 compared to GS2 (Table 4).

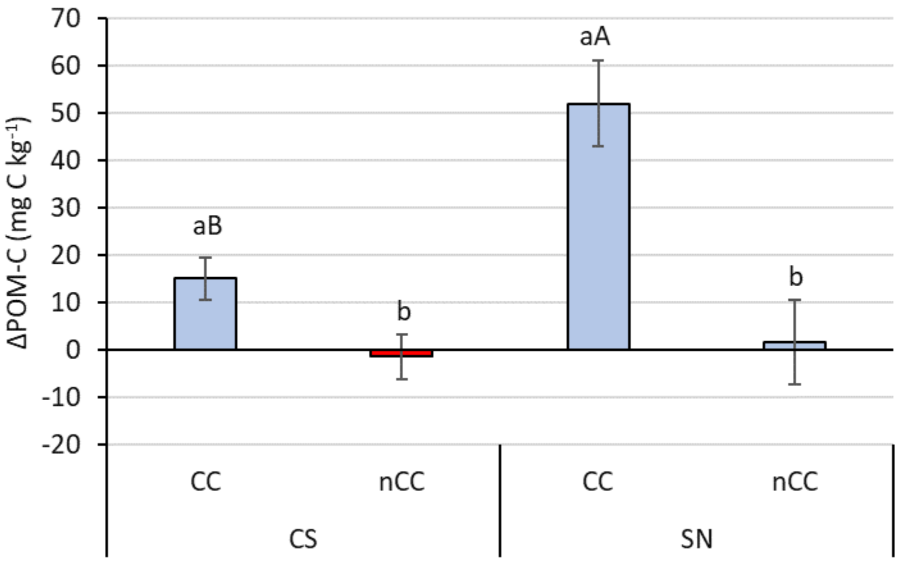

Figure 4 Effect of Loc (CS vs SN) and ground cover (CC vs nCC) interaction on the variation of soil Particulate Organic Matter-Carbon (ΔSOC). Error bars indicate standard error. Lowercase letters represent significant differences between GC Uppercase letters specify significant differences between Loc according to the Tukey HSD test.

The ΔPOX-C was significantly affected by the interaction Loc x GC (Table 3). At SN, the value of ΔPOX-C was significantly increased by CC, leading to 7.7 mg C kg^{-1} more than those in nCC (Fig. 5). Moreover, with CC, ΔPOX-C in SN was approximately 32 times higher than in CS. Overall, the variance in ΔPOX-C was primarily explained by two-way interactions (35%), ground cover (32%), and location (18%) (Fig. S1).

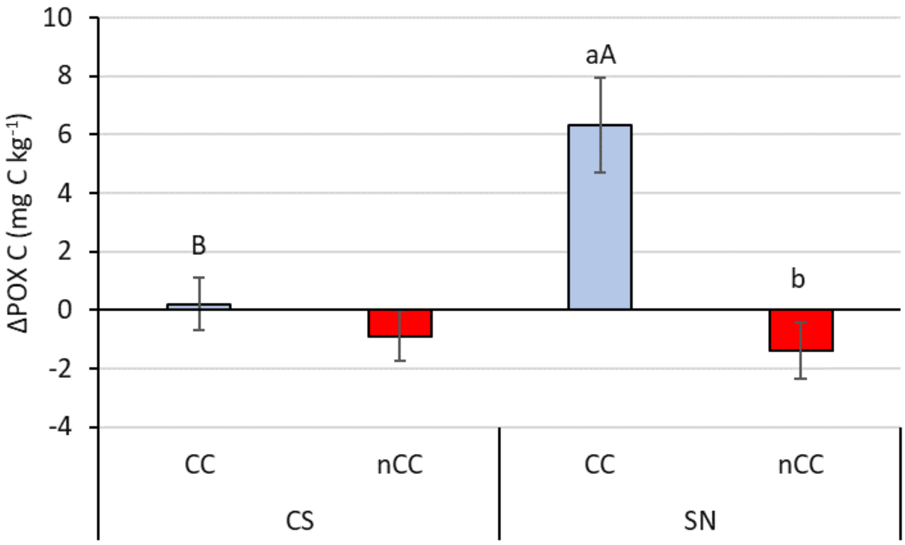

Figure 5 Effect of Loc (CS vs SN) and ground cover (CC vs nCC) interaction on the variation of soil permanganate-oxidable carbon (ΔPOX-C). Error bars indicate standard error. Lowercase letters represent significant differences between GC, while uppercase letters specify significant differences between Loc according to the Tukey HSD test.

Regardless the considered interactions, ΔMBC values were consistently higher in CC than in nCC. The effect of Loc X GC X SDe on ΔMBC was significant (Table 3). The ΔMBC values were significantly higher in CC compared to nCC at both CS and SN, regardless of soil layer (Fig. 6a). With CC at CS, the ΔMBC measured in the 10-20 cm layer was significantly higher by 18.3% than in the 5-10 cm layer, but not significantly different from the 0-5 cm layer. Conversely, at SN with CC, the ΔMBC value in the 0-5 cm layer was significantly higher by 51% compared to the 5-10 cm layer, which, in turn, was higher by 65% than the 10-20 cm layer. With CC, while ΔMBC in the upper 5 cm was significantly higher by 17% at SN compared to CS, this trend was reversed in the deeper layers: in the 5-10 cm layer, ΔMBC measured at CS exceeded that at SN by 22%, and in the 10-20 cm layer, the value at CS was more than double that observed at SN.

Another three-way interaction (GS X Loc X GC) had a significant effect on ΔMBC (Fig. 6b). During GS1 within CC, ΔMBC measured at CS was significantly higher by 42.3% compared to SN. The overarching variance in ΔMBC was primarily attributed to the GC factor, which explained 73.7% of the total variance (Fig. S1). In general, ΔMBC values were significantly higher at CS compared to SN (Table 4).

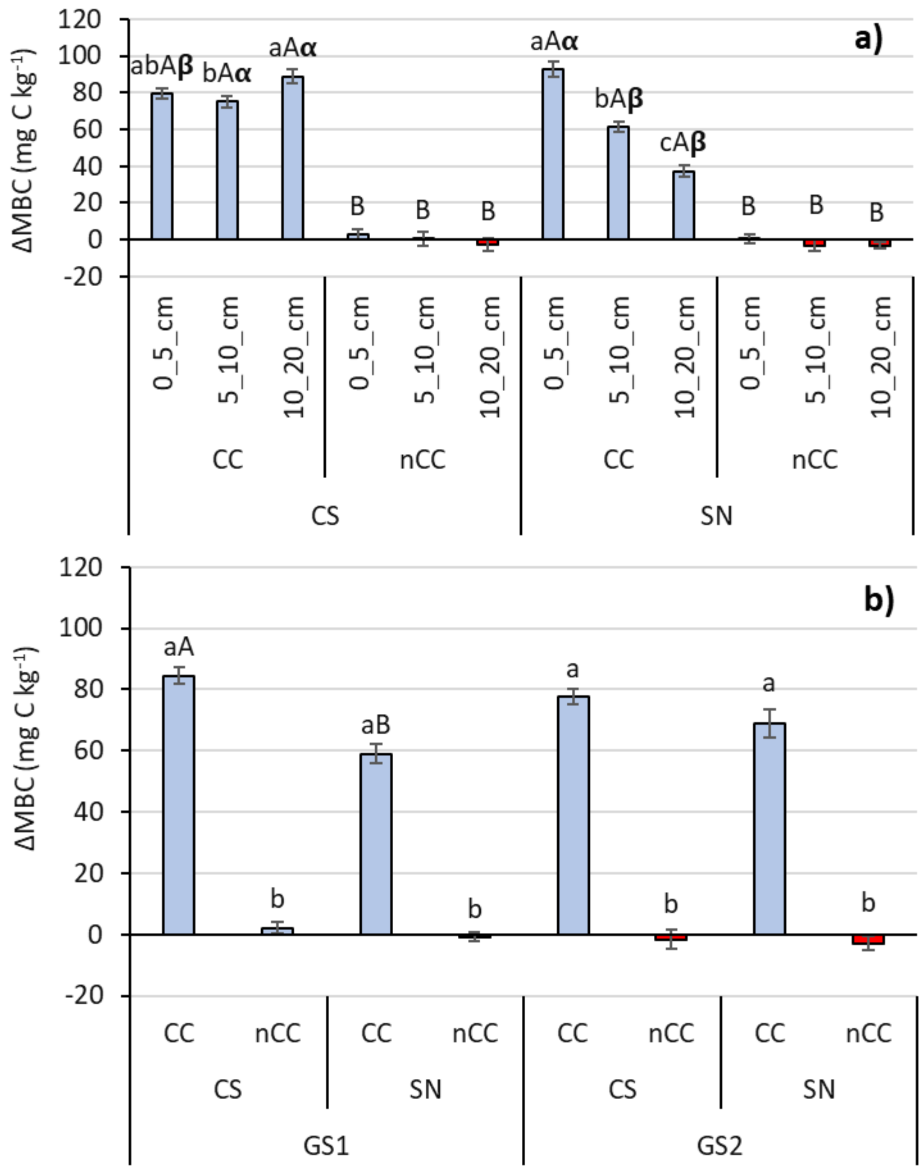

Figure 6 (a) Effect of the interaction between location (CS vs SN), ground cover (CC vs nCC), and soil depth (0-5, 5-10,10-20 cm) on the variation of soil microbial biomass carbon (ΔMBC). **(b)** Effect of the interaction between growing season (GS1 vs GS2), location (CS vs SN), and ground cover (CC vs nCC) on the variation of soil microbial biomass carbon (ΔMBC). Error bars indicate standard error. Lowercase, uppercase and Greek letters indicate significant differences between levels of the same factor according to the Tukey HSD test. Lowercase letters represent significant differences between soil depth, while uppercase letters specify significant differences between ground cover. Greek letters indicate significant differences between locations.

6.4.3. Effect of the analyzed factors in reduced tillage on the soil phosphorus dynamics

The ΔOls P was significantly influenced by Loc × GC × SDe (Table 3). Significant differences in ΔOls P between soil layers were observed exclusively at SN both within CC and nCC (Fig. 7). With CC, the ΔOls P measured in the 0-5 cm layer was approximately five times higher than that in the 10-20 cm layer, while the value in the 5-10 cm layer was similar to the other layers. Conversely, at SN under nCC, the ΔOls P measured in the 10-20 cm layer was significantly higher by 75% compared to the 0-5 cm layer, with the 5-10 cm layer showing no significant difference from the others. In the 5-10 cm layer at CS, the value of ΔOls P measured in CC was significantly higher by 0.16 mg P kg^{-1} compared to nCC. At SN, the ΔOls P values measured in CC in the 0-5 cm and 5-10 cm layers were approximately 8 and 2 times higher, respectively, than those in nCC. In the 0-5 cm layer within CC, the ΔOls P value measured at SN was three times higher than that at CS. Overall, a significant increase in ΔOls P was observed in CC compared to nCC and in the 0-5 cm layer compared to the 5-10 cm and 10-20 cm layers (Table 4). The variance in ΔOls P was primarily explained by two-way interactions (32.4%), followed by SDe (29.3%), GC (16.2%), three-way interactions (15.2%), and Loc (4.7%) (Fig. S1).

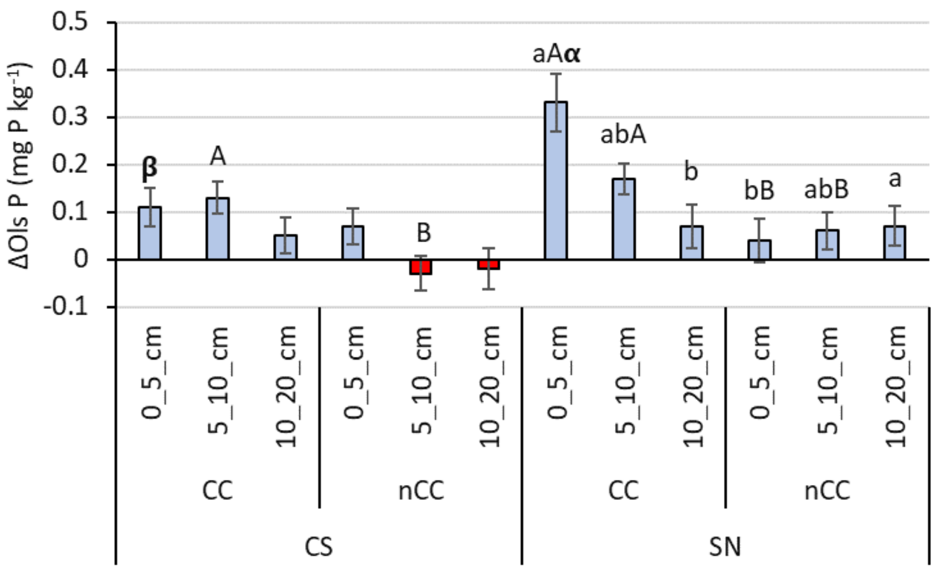

Figure 7 Effect of the location X ground cover X soil depth (Loc X GC X SDe) on the variation of soil Olsen phosphorus (ΔOls P). Error bars indicate standard error. Lowercase, uppercase and Greek letters indicate significant differences between levels of the same factor according to the Tukey HSD test. Lowercase letters indicate significant differences between soil depth within Loc and GC, while uppercase letters specify significant differences between GC. Greek letters specify significant differences between Loc

The Loc × GC x SDe interaction had a significant effect on ΔMBP (Table 3). Across all conditions, ΔMBP values were consistently higher in CC than in nCC, irrespective of location or soil depth (Fig. 8a). WithiCC at SN, ΔMBP measured in the 0-5 cm layer was significantly higher by 42.7% and 60.8% compared to the 5-10 cm and 10-20 cm layers, respectively. Notably, within the 0-5 cm layer under CC, ΔMBP at SN exceeded that at CS by approximately 38%. Conversely, in the 5-10 cm layer under CC, ΔMBP at CS was significantly higher by 25.3% than that observed at SN. The ΔMBP was also influenced by the three-way interaction GS × Loc × GC (Table 3). Consistently, ΔMBP values were higher in CC compared to nCC across all GS and Loc combinations (Fig. 8b). During GS2 within nCC, ΔMBP measured at SN was significantly higher by 0.53 mg P kg^{-1} than that observed in CS. In contrast, within nCC at CS, ΔMBP measured during GS1 exceeded that in GS2 by 0.24 mg P kg^{-1}. Conversely, within nCC at SN, ΔMBP measured during GS2 was significantly higher by 0.5 mg P kg^{-1} compared to GS1. Overall, the ΔMBP variance was explained by GC, accounting for 67.6% of the total variance (Fig. S1).

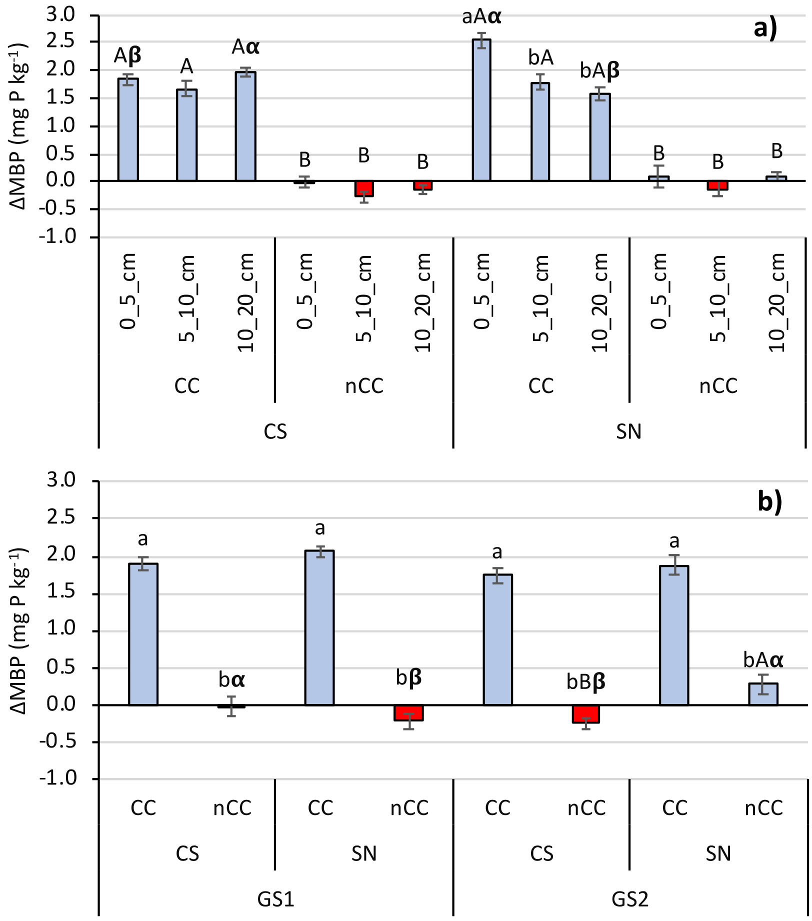

Figure 8 (a) Effect of the interaction between location (CS vs SN), ground cover (CC vs nCC), and soil depth (0-5, 5-10, 10-20 cm) on the variation of soil microbial biomass phosphorus (ΔMBP). **(b)** Effect of the interaction between growing season (GS1 vs GS2), location (CS vs SN), and ground cover (CC vs nCC) on the variation of soil microbial biomass phosphorus (ΔMBP). Error bars indicate standard error. Lowercase, uppercase and Greek letters indicate significant differences between levels of the same factor according to the Tukey HSD test. Lowercase letters represent significant differences between ground cover, while uppercase letters specify significant differences between locations. Greek letters indicate significant differences between growing seasons.

6.4.4. Effect of the analyzed factors in reduced tillage on the soil nitrogen dynamics

The ΔNtot was significantly influenced by the two-way interaction Loc × GC (Table 3). At SN, the ΔNtot value was significantly higher by 34 mg N kg^{-1} compared to nCC (Fig. 9). Within CC, the ΔNtot value observed at SN was significantly higher by 17.8 mg N kg^{-1} compared to CS. In nCC, the ΔNtot value measured at CS was found to be significantly higher by about 15 mg N kg^{-1} compared to SN. Overall, the ΔNtot value in GS1 was significantly higher than in GS2, as was the ΔNtot value in CC compared to nCC (Table 4). Two-way interactions accounted for 49% of the total variance of ΔNtot, followed by GC with 30.6% and GS with 16.3% (Fig. S1).

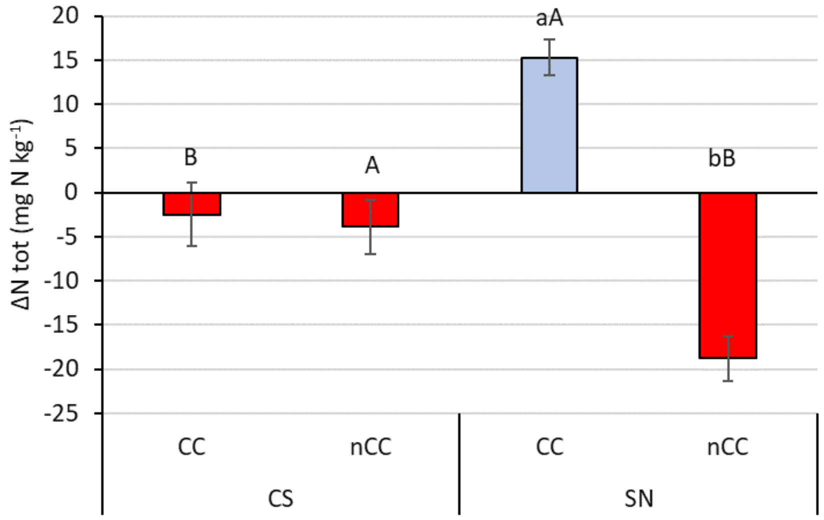

Figure 9 Effect of location (CS vs SN) and ground cover (CC vs nCC) interaction on the variation of soil total nitrogen (ΔN tot). Error bars indicate standard error. Lowercase letters represent significant differences between ground cover, while uppercase letters specify significant differences between locations according to the Tukey HSD test.

Regarding ΔMBN, approximately 71% of the total variance was explained by GC (Fig. S1). The three-way interaction Loc × GC × SDe showed also a significant effect on ΔMBN (Table 3). The values of ΔMBN were consistently higher in CC compared to nCC, regardless of location or soil depth (Fig. 10). Specifically, at CS, ΔMBN values in the 0-5 cm, 5-10 cm, and 10-20 cm layers were significantly higher in CC than in nCC by 5.92, 7.72, and 6.29 mg N kg^{-1}, respectively. Similarly, at SN, ΔMBN values in CC were significantly higher than those in nCC by 7.61, 4.11, and 3.17 mg N kg^{-1} in the 0-5 cm , 5-10 cm , and 10-20 cm layers, respectively. Under nCC at CS, ΔMBN measured in the 5-10 cm layer was significantly lower by 2.12 mg N kg^{-1} compared to the 0-5 cm layer, while the value in the 10-20 cm layer was not significantly different from the others. At SN with CC, ΔMBN measured in the 0-5 cm layer was significantly higher—by 93%—than that in the 10-20 cm layer. No significant variations in ΔMBN were detected between the 5-10 cm layer and the others at SN under CC. Overall, a significant increase of ΔMBN was showed at CS compared to SN, and in CC compared to nCC (Table 4). The remaining total variance was explained by two-way interactions and Loc, which accounted for 13.8% and 11% of the total variance, respectively (Fig. S1).

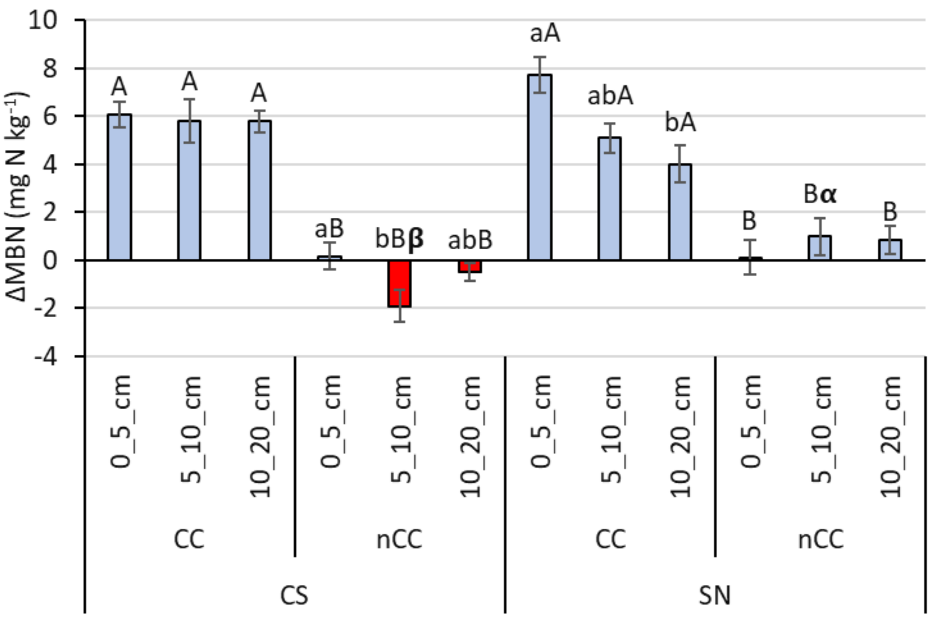

Figure 10 Effect of the interaction between location (CS vs SN), ground cover (CC vs nCC), and soil depth (0-5, 5-10, 10-20 cm) on the variation of soil microbial biomass nitrogen (ΔMBN). Error bars indicate standard error. Lowercase, uppercase and Greek letters indicate significant differences between treatment according to the Tukey HSD test. Lowercase letters represent significant differences between soil depth, while uppercase letters specify significant differences between ground cover. Greek letters indicate significant differences between locations.

6.5. Discussion

6.5.1. Effect of Location and Growing Season on the Biomass and phosphorus accumulation of tillage radish

Tillage radish (TR) showed different performance under different cropping systems and pedoclimatic conditions as revealed by the effects of location (Loc) and growing season (GSs) on the biomass yield of TR, as well as on its phosphorus uptake. Biomass yield was the highest in the site with the lowest sand content and pH value (SN). This texture may have facilitated better root growth and nutrient uptake compared to the clay loam soil (CS), which is more prone to compaction and waterlogging under reduced tillage (Van den Putte et al. 2010). A lower pH likely enhanced nutrient uptake and microbial activity, supporting TR growth (White and Weil 2011; Wendling et al. 2016). Higher temperatures and early rainfall at SN during the TR growth period likely enhanced its establishment and reduced stress (Toom et al. 2019). This agrees with White and Weil (2011), who observed similar TR yields under conventional tillage than in SN; however, they observed higher yields under no-till than in CS.

The increased P uptake at SN, where TR exhibited better growth, compared to CS, where growth was restricted, reveals the role of site-specific factors in influencing nutrient dynamics and availability to plants. The lower pH at SN likely created a more favorable environment for nutrient availability and microbial activity, which are critical for phosphorus uptake (White and Weil 2011; Wendling et al. 2016). Toom et al. (2019) observed a comparable trend for P uptake by TR across two different growing seasons. Additionally, Wendling et al. (2016) reported total P uptake by daikon radish under conventional tillage of 20 kg P ha^{-1}, similar to those observed in GS1 at SN in this study (about 18 kg P ha^{-1}). This suggests that TR at SN experienced favorable conditions for development, even under reduced tillage. Conversely, P uptake by TR at CS was considerably lower, reflecting the growth restriction due to sub optimal conditions in this location. In our study, the P concentration observed in radish tissues was lower compared to that reported by Wendling et al. (2016), which appears closely linked to the limited P availability in both of our experimental soils. Consistently, the highest P concentration in radish tissues was recorded at SN, where the soil's available P content was slightly higher than in CS, supporting the hypothesis that soil P availability strongly influenced plant uptake. These findings highlight the significance of location-specific conditions in determining TR performance, emphasizing the need for tailored management practices to enhance nutrient uptake efficiency and growth under suboptimal conditions.

6.5.2. Effect of tillage radish on different organic carbon fractions

The increase in SOC was consistently higher in CC than in nCC, particularly at SN where TR biomass accumulation was greatest. This reflects the broader trend across all studied soil parameters, where greater biomass and active root systems enhanced nutrient cycling, organic matter dynamics, and microbial activity (Blanco-Canqui et al. 2015; Frasier et al. 2016). TR’s contribution to SOC appears effective even below the commonly cited biomass threshold of 2 t ha^{-1} (Blanco-Canqui et al. 2024), likely due to its rapid decomposition, deep taproot, and efficient residue incorporation (Wang et al. 2017; Zhang et al. 2022; Inagaki et al. 2021; Torabian et al. 2025). Conversely, limited SOC gains at CS may result from lower TR biomass, potentially due to soil compaction under reduced tillage (Van den Putte et al. 2010), and less favorable climatic conditions (Blanco-Canqui et al. 2015). A similar trend was observed for POM-C, which increased more at SN, reinforcing the central role of biomass input in driving labile carbon fractions (Derrien et al. 2023; Zhang et al. 2022; Rubio et al. 2025). The smaller change at CS further underscores the influence of environmental conditions, such as temperature and moisture, on decomposition dynamics and carbon stabilization (Frasier et al.

2016). The trend of the other labile fraction of soil carbon studied (POX-C), with significant increase in SN and not detectable change in CS also highlights the importance of biomass input in enhancing labile soil organic C fractions. While POX-C has traditionally been associated with fresh organic matter inputs, recent literature has clarified that it more accurately represents a pool of organic carbon that is partially microbially processed and partially stabilized (Chambers et al. 2024; Christy et al. 2023), rather than newly added, highly labile compounds (Rubio et al. 2025). This understanding suggests that very recent inputs-especially those rich in simple sugars-may not immediately impact POX-C levels, as they require microbial transformation before becoming detectable in this fraction. In this context, the significant increase at SN may reflect not only greater biomass input from TR but also more favorable conditions for microbial processing and stabilization. Wang et al. (2017) similarly reported that TR significantly increased POX-C values compared to the fallow systems, likely due to its rapid residue decomposition and nutrient release. Frasier et al. (2016) found that soil temperature and moisture directly affect residue decomposition rates, which could contribute to explain the negligible increment in POX-C observed at CS. MBC was also consistently higher in CC across locations and depths, highlighting the microbial benefits of TR-derived residues (Torabian et al. 2025). At CS, greater MBC at 10–20 cm suggests deep taproot decomposition (Hu et al. 2018; Wang et al. 2017), while at SN, increases in surface MBC likely stemmed from aboveground biomass moderating temperature fluctuations and stimulating microbial activity (Frasier et al. 2016).

6.5.3. Effect of tillage radish on soil phosphorus

A notable stratification of Olsen P was observed at SN, especially under CC. With CC, the highest increase in Olsen P was recorded in the 0-5 cm layer, suggesting that TR may have remobilized phosphorus from the deeper soil layers through root activity. Similar increases of soil P test were observed by Solangi et al. (2024) after the cultivation of some brassica species, which are known for their ability to mobilize P via root exudates and enzymatic activity. Under nCC at SN, an opposite trend was observed, with the 10-20 cm layer showing significantly higher ΔOls P than the 0-5 cm layer. This suggests a redistribution of phosphorus deeper into the soil profile, likely due to the absence of TR contributions, a pattern commonly reported in conservation systems (Smith et al. 2017; Richardson and Simpson 2011). At CS, in the 5-10 cm layer, the increase in Olsen P was significantly higher in CC compared to nCC, albeit with a relatively small increase (0.16 mg P kg^{-1}), suggesting that even in less favorable conditions, TR could contribute to modest improvements in P availability to crops.

Regarding the microbial biomass P (MBP), this study confirms the substantial positive effect of TR on enhancing microbial biomass and activity. Similar trends were observed with MBC, suggesting that residue inputs and soil aeration near the surface likely stimulated microbial activity in the location with the highest biomass yield. Tillage radish likely mobilizes soil P and accumulates it in root tissues, which is transferred into the microbial biomass after decomposition (Wang et al. 2021; Richardson and Simpson 2011). This P pool becomes available to plants following microbial biomass decay (Richardson and Simpson 2011; Sugito et al. 2011), underscoring the dual role of TR in nutrient cycling and plant availability. This can contribute to explain the increase in Olsen P after TR. Environmental conditions, including rainfall and soil aeration, could also have played a role in MBP dynamics. At SN during GS2, lower rainfall likely improved soil aeration, resulting in higher ΔMBP compared to GS1 as supported by Gross et al. (2018), who noted that better aeration enhances microbial activity. Conversely, at CS, the value of MBP remained relatively stable during GS1 but slightly declined during GS2, probably because the lower rainfall occurred in GS2 may have constrained microbial growth and activity. These findings align with Frasier et al. (2016), who reported that moisture availability and residue quality are critical for maintaining microbial P dynamics under reduced tillage systems.

6.5.4. Effect of TR on soil nitrogen

Overall, TR increased more total N in soil in SN compared to fallow, and this difference was higher in SN than in CS. This highlights not only the contribution of TR to N accumulation in soil but also the critical role of site-specific conditions (management and environmental) in N dynamics. At SN, soil sampling was conducted after TR termination and frost killing, when most of the nitrogen initially absorbed by TR had likely been released into the soil. In contrast, at the CS site, TR was still alive during the second sampling, meaning that a substantial portion of nitrogen was still retained within the plant biomass. The effect of TR indicates a trend toward N conservation associated with TR as cover crop. This is likely achieved through N uptake by cover crop, which reduces leaching, and subsequent N accumulation through biomass input in soil after TR termination as observed by Vogeler et al. (2019). This promotes an efficient nitrogen cycling. Thus, TR cultivation may serve as a valuable strategy for achieving more sustainable N management practices. Recently, Elhakeem et al. (2023) also highlighted the ability of radish as cover crops to reduce N leaching, further supporting N conservation. The effect of cover crops in reducing leaching depends on biomass production and root activity (Blanco-Canqui et al. 2015), which is consistent with the biomass-driven differences previously discussed. Frasier et al. (2016) noted that greater residue inputs not only enhance N retention but also promote microbial activity, which plays a key role in stabilizing nitrogen in the soil. On the other hand, due to the high nitrate content of radishes (Bahadoran et al. 2016), the mineral N scavenged by TR could be lost through leaching, particularly under conditions of high rainfall, which are common in late winter and early spring in Tuscany (Fabbri et al. 2025). There, the sowing of spring cereal crops typically occurs from the end of February to the end of March, further influencing the timing and potential risk of nitrate leaching between TR termination and the sowing of subsequent crop. Nitrogen immobilization in microbial biomass can temporarily mitigate such risks (Kuzyakov and Xu 2013). Another promising strategy to reduce nitrate losses could be the cultivation of radish together with winter-hardy species as cover crops (Elhakeem et al. 2023), which can recover the nitrates released by TR residues. This dual role of TR, enhancing N retention while posing potential later leaching risk, necessitates careful management practices, including optimized residue handling and strategic use of TR as a cover crop, to maximize its benefits while minimizing losses.

The consistent higher increase of MBN in CC compared to nCC, irrespective of location and soil layer, underline the significant role of TR cultivation in enhancing microbial N storage. The increase in MBN is attributed to the decomposition of TR residues, which provide a steady N source for microbial communities. Jensen et al. (1997) reported similar finding with oilseed rape residues, highlighting that brassica residues release N gradually, supporting microbial growth and activity over time. This aligns with findings for MBC and MBP, further reinforcing that TR cultivation promotes overall microbial biomass and nutrient cycling. Microbial nitrogen cycling is critical for maintaining soil fertility and organic matter stability (Kuzyakov and Xu 2013). The presence of TR residues, particularly in CC treatments, may also stimulate microbial community diversity and activity, creating a more resilient nitrogen cycle by promoting the stabilization of nitrogen through enhanced microbial interactions (Wang et al. 2021). These interactions can lead to improved nitrogen retention in the soil, reduced losses through leaching, and more efficient nitrogen transformations, ultimately supporting soil fertility and ecosystem sustainability (Li et al. 2025). Environmental factors, such as soil moisture and aeration, likely contributed to the observed differences in the increment in MBN between both locations. Frasier et al. (2016) noted that moisture availability and residue quality significantly affect microbial activity, with higher residue input and favorable conditions enhancing microbial N storage in soil.

This study highlights the role of tillage radish (TR) residues in influencing soil properties, with location-specific conditions playing a significant role. At SN, where soil texture and pH were more favorable, TR showed better growth, leading to higher phosphorus uptake and enhanced microbial activity compared to CS, where compaction and less favorable conditions limited TR performance. The positive effects on soil organic carbon (SOC) and microbial biomass carbon (MBC) were more pronounced at SN, emphasizing the importance of biomass input for nutrient cycling. In terms of nitrogen dynamics, TR improved nitrogen retention and microbial nitrogen storage, especially at SN. However, the potential risk of nitrate leaching, particularly in high rainfall periods, underscores the need for careful management of TR termination timing. Overall, the findings suggest that the benefits of TR as a cover crop are closely linked to local conditions, and management practices should be adapted to optimize its contributions to soil health and sustainability.

6.6. Conclusions

Tillage radish as cover crop instead fallow led to enhanced soil health indicators related to C, N, and P cycling in the two studied locations with different soil types, climatic conditions, and cropping systems. The cover crop increased soil organic C, labile C fractions, Olsen P, total N, and microbial biomass in soil. Also, tillage radish showed a potential to reduce N losses. This enhancement was more evident under conditions leading to higher biomass accumulation and phosphorus (P) uptake by tillage radish. These findings underscore the positive effects of tillage radish as cover crop on C storage, microbial biomass, and N and P availability and cycling which varied under different pedoclimatic conditions. To better harness the benefits of tillage radish, future studies should confirm these effects in multiple growing seasons, particularly in agroecosystems with diverse management practices and environmental conditions.

6.7. Acknowledgments

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7. Fourth paper

Integrating multiple sustainable practices in Cropping Systems: A Field Study in the Mediterranean Region

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7.1. Abstract

European policies increasingly encourage the integration of multiple sustainable cropping practices to enhance the environmental resilience and agricultural sustainability. However, evidence of their short-term combined effects within conventional systems remains limited. In the present study, a three-year field experiment was implemented to evaluate the integrated deployment of tillage radish cover cropping, red clover-driven crop rotation, olive and hazelnut alley cropping, and conservation tillage in a traditional cereal monocropping context. The objective was to assess their combined effects on crop performance and soil health indicators. Results revealed that sowing TR before spring barley significantly increased grain and protein yields by approximately 27% and 29%, respectively, compared to fallow management. In the early transition phase to reduced tillage, the energy yield of harvestable crops decreased by up to 72%, but this decline was mitigated by the adoption of crop rotation. After three years, the combination of an olive-based alley cropping system and diversified rotation produced an herbaceous crop protein yield (~2 t ha^{-1}) comparable to the monoculture system. From a soil perspective, tillage and CC management emerged as key drivers of soil organic carbon dynamics. Under plough tillage, the increase in soil organic carbon was 52.9% higher than under reduced tillage, while plots with cover crops showed a 68.7% greater soil organic carbon increase compared to fallow plots. This research highlights the potential of combining multiple sustainable practices to improve crop performance and soil health in the short-term.

7.2. Introduction

Current European policy emphasizes the transition toward sustainable agriculture, as the sector contributes to approximately one-third of total greenhouse gas emissions (Crippa et al. 2021). Agricultural systems, both globally and in Europe, have undergone significant transformations over the past half a century. They have steadily evolved into highly simplified frameworks dominated by monocultures, continuous monocropping, and extended fallow periods (Restovich et al. 2022). These systems are also characterized by a significant reliance on fossil energy for inputs, encompassing fertilizers, pesticides, and fuel. As a result, current agricultural practices have rendered 60-70% of European Union (EU) soils unhealthy (Veerman et al. 2020).

To address these challenges, several solutions have been proposed, such as the intensification and diversification of cropping systems, as well as the reduction of tillage intensity. However, the short-term effects of incorporating these strategies into conventional systems (characterized by monocropping, reliance on fallow periods, and intensive plough-based tillage) remain insufficiently investigated.

In some parts of the Mediterranean region, the fallow periods may extend up to nine months between the cereal harvest and the sowing of the subsequent crop (Leoni et al. 2022). During this period, fields are typically left uncultivated, leading to prolonged soil exposure. This prolonged fallow exacerbates soil degradation, particularly in Mediterranean environments, where water availability is a key constraint to crop productivity (Simon-Miquel et al. 2024; Ruis et al. 2023). Therefore, fallow represents both a challenge and an opportunity for the improvement of conventional cropping systems. The concept of cropped land intensification was first reported by Boserup (1965), and referred to either the reduction or elimination of the fallow period, as well as the more intensive use of environmental resources through sequential double cropping and crop-pasture rotation (Novelli et al. 2013).

Cropland intensification can be achieved through the use of cover crops (CCs) which are fast-growing species that are cultivated between periods of cash crop production (SSSA, 2008). These crops are generally used to provide multiple benefits, including soil improvement and the provision ecosystem services (Blanco-Canqui et al. 2015). Replacing fallow with CCs has already shown potential in reducing greenhouse gas emissions (Manalil et al. 2014) and in increasing nitrogen (N) availability for subsequent crops (Nascimento et al. 2024). Additional short-term benefits of CCs include increased phosphorus (P) bioavailability, as CCs can promote the conversion of P from low-availability pools into more labile forms within the rhizosphere, particularly in P-depleted soils (Maltais-Landry et al. 2014). Tillage radish (TR) is one such cover crop that has shown promise in increasing soil P test levels (White and Weil, 2011, Pescatore et al. 2025a; Pescatore et al. 2025b). However, the effects of TR on subsequent crop performance and overall system profitability require further investigation. The use of TR as a CC has been tested within conservation agriculture systems. In these systems, soil compaction often poses a potential limitation to cropping system profitability (Vanderhasselt et al. 2024), particularly during the early years of transition from conventional tillage.

The benefits of cropping system diversification, through the inclusion of legumes in cereal-based cropping systems, on crop performances and profitability have already been demonstrated (Tzemi et al. 2024). The primary contribution of legumes to such systems is their ability to fix atmospheric nitrogen (N), thereby reducing the need for synthetic N input and enhancing the growth of subsequent crops (Preissel et al. 2015). However, N enrichment is not the sole advantage of breaking cereal monoculture with legumes (Geng et al. 2023). Legumes can also act as a source of organic matter for the soil, even in the short-term (Loges et al. 2024), thereby improving the chemical and physical properties of the soil for the cultivation of the subsequent cereal crop (McCallum et al. 2004). Therefore, several studies have reported that the integration of legumes into a cropping system can increase the average yield of the subsequent cereal crop by approximately 24% compared to continuous monocropping (Kirkegaard et al. 2008).

Even though the benefits of legumes and other diversification strategies have been well documented in temperate regions (Reckling et al. 2022), it is hypothesized that the integration of these practices in combination with other diversification strategies may positively affect crop performances, soil properties and overall system performance under Mediterranean conditions in the short-term.

In the context of reduced tillage, diversification of the cereal-based cropping system could also alleviate the drawbacks associated with the transition to conservation agriculture. For instance, the root apparatus of legumes can develop effectively even in compacted soil layers (Głąb 2015), a common feature in the early phases of conversion to conservation practices (López-Garrido et al. 2014).

Alley cropping systems also represent a promising strategy for diversifying and intensifying cropping systems. Olive groves, a major Mediterranean crop, cover more than 4 million hectares (EUROSTAT 2020). During the first six years of olive tree establishment, when yields are minimal (Rezgui et al. 2024), land use efficiency can be optimized by intercropping with herbaceous species. Likewise, hazelnuts experience a prolonged juvenile phase, during which intercropping can be implemented to enhance land productivity. Hazelnut cultivation is of particular interest to Italy as the world's second-largest producer (FAOSTAT 2023).

This approach enables the cultivation of additional crops, thereby maximizing land use and productivity during periods that would otherwise remain unproductive. Furthermore, such systems were also shown to improve soil health in a hazelnut-based system (Matisic et al. 2023). Zuazo et al. (2020) demonstrated that the integration of sustainable management practices (such as reduced tillage intensity) significantly enhanced soil health in Mediterranean olive orchards compared to conventional systems, even in the short term. Similarly, the integration of diverse and intensive cropping systems into young high-density plantations may provide benefits for various soil properties. Studies have reported that intensive olive groves within agroforestry systems hold strong potential for increasing soil organic carbon stocks under Mediterranean conditions (Zayani et al. 2023). Therefore, the inclusion of diversified and intensified herbaceous cropping systems in young intensive orchards may simultaneously enhance soil properties and optimize land use during the early unproductive periods of orchard establishment.

To the best of our knowledge, few studies have investigated the early-stage effects of integrating multiple sustainable practices into a conventional cereal monocropping system. In particular, crop performance and soil properties have not been adequately considered. The combination of different practices has the potential to generate synergistic benefits while mitigating the limitations associated with their individual implementation (Tscharntke et al. 2012).

This study aims to contribute to the transition towards more sustainable Mediterranean cropping systems by identifying optimal combinations of sustainable practices. Specifically, we evaluated the effects of:

(i) replacing fallow with tillage radish (Raphanus sativus var. longipinnatus L.H.Bailey) as a cover crop;

(ii) breaking the cereal monocropping cycle with red clover (Trifolium pratense L.),; and

(iii) introducing two alley cropping systems that integrate olive (Olea europaea L.) and hazelnut (Corylus avellana L.) orchards with the herbaceous crops.

These systems were assessed under both conventional and reduced tillage to explore opportunities for intensifying and diversifying traditional cereal-based monocropping. We hypothesized that the integration of these practices would enhance crop performance and ecosystem services while improving soil health and increasing overall system profitability in a Mediterranean cereal-based system

7.3. Materials and methods

7.3.1. Site description

The experiment was carried out at the Tereto Living Lab (TLL; Marciano della Chiana, Arezzo, Italy; 43.3179° N, 11.7952° E, 246 m a.s.l.) from August 2021 to July 2024 under rainfed conditions. According to the Soil Survey Staff (2014), the soil is classified as a Typic Vertic Calciustepts, with a clay loam texture and alkaline pH (Table 1). The climate is classified as hot-summer Mediterranean (Cfa) under the Köppen system. This is characterized by hot, dry summers, with the majority of rainfall occurring in autumn.

Table 1 Soil properties determined before the starting of the trial for the upper 15 cm soil layer. The results are expressed as the mean values with standard deviations

Soil property	Value ± SD
Sand (%)	32.82 ± 1.81
Silt (%)	32.24 ± 0.94
Clay (%)	34.94 ± 2.05
pH_w (1:5)	8.20 ± 0.03
EC (mS cm^{-1})	0.20 ± 0.01
CEC (meq 100 g^{-1})	28.97 ± 1.44
$CaCO_3$ (%)	5.62 ± 0.17
Soil Organic Carbon (%)	1.00 ± 0.55
Total Nitrogen (%)	0.10 ± 0.06
Olsen P (mg P kg^{-1})	7.90 ± 0.56

pH_w= pH in 1:5 soil/water extract; CEC= cation exchange capacity; EC= electrical conductivity in 1:2.5 soil/water extract; Olsen P= Available Phosphorus was determined using the method described by Olsen et al. (1954)

7.3.2. Experimental design

The experimental field was subdivided into two sections to differentiate soil tillage management (TM) approaches. One section was managed under conventional tillage (moldboard ploughing to a depth of 30 cm, plough) and the other under reduced tillage (chisel ploughing to 30 cm; reduced) (Figure 1). Two main cropping sequences (CSs) were arranged in a strip-plot design, comparing continuous cereal cropping (CONTIN) with a cereal-legume rotation (ROT). Ground cover management (GCM) was arranged in a split-plot design, comparing fallow (no cover crop sowing; nCC) with the sowing of CC using TR.

Each plot measured 15 × 15 m (225 m^2). On one edge of each plot, aligned along the north–south axis, a row of trees was planted to implement the alley cropping (AC) configuration. The AC treatment consisted of three levels: tree rows planted with olive trees (Oliv), tree rows planted with hazelnut trees (Haz), and a control condition with no trees, represented by a furrow typically found in conventional monoculture systems (Mono). To assess the local effect of the AC treatment on soil and crop properties (referred to as the ACe treatment), soil and plant samples were collected in triplicate at two positions. These positions were 1 meter from the furrow or the tree row (Border) and at the centerline of the plots, which served as the control area (no AC effect; Center). Block orientation was established based on preliminary measurements of soil variability

7.3.3. Cropping system management and field operations

Prior to the experiment, the TLL field was cultivated over five years using an alternating sequence of sunflower (Helianthus annuus, L.) and winter wheat. In particular, the crop preceding the establishment of the experiment was durum wheat (Triticum turgidum subsp. Durum Desf). The experiment was initiated in August 2021. In March 2022, at the point coinciding with the planting of the trees, 3-meter-wide soil strips were ripped to a depth of 0.7 m to prepare for tree planting.

Olive (Olea europaea L., cv. Lecciana, Agromillora Group, Barcelona, Spain) and hazelnut (Corylus avellana L., cv. Tonda Francescana, Foundation for Agricultural Education, Perugia, Italy) trees were selected for their suitability for high-density planting and mechanized management. The intra-row spacing was approximately 1.5 m for olive and 2 m for hazelnut. Wide 30 m alleys were maintained between tree rows to accommodate the herbaceous crops (Figure 1) and to enhance system diversity and land use efficiency.

Table S1 Timeline of field activities during the three growing seasons (2021-2022, 2022-2023, and 2023-2024 referred to as GS1, GS2, and GS3, respectively)

	GS1	GS2			GS3	
	Winter Wheat	**Tillage Radish**	**Spring Barley**	**Red clover**	**Tillage Radish**	**Spring Barley**
Primary Tillage	October 6th, 2021	August 5th, 2022	February 10th, 2023	February 10th, 2023	August 29th, 2023	February 2nd, 2024
Secondary Tillage	November 25th, 2021	September 9th, 2022	February 13th, 2023	February 13th, 2023	September 11th,2023	February 2nd, 2024
Phosphorus supply	December 3rd, 2021	NA	February 16th, 2023	February 16th, 2023	NA	February 5th, 2024
Sowing	December 17th, 2021	September 14th, 2022	February 21st, 2023	March 24th, 2023	September 16th, 2023	February 8th, 2024
Nitrogen supply 1	February 23rd, 2022	NA	March 24th, 2023	NA	NA	March 13th,2024
Nitrogen supply 2	April 5th,2022	NA	April 20th, 2023	NA	NA	April 3rd, 2024
CC termination	NA	February 2nd, 2023	NA	NA	January 31st, 2024	NA
Harvesting	July 11th ,2022	NA	July 17th, 2023	July 13th, 2023	NA	July 12th, 2024

Field operations for herbaceous crops, including exact dates, are reported in Table S1. The first growing season (GS1) spanned from the sowing to the harvesting of winter wheat. The second growing season (GS2) covered the period from the sowing of tillage radish to the harvesting of spring barley and red clover. The third growing season (GS3) covered the period from the sowing of tillage radish, to the harvesting of spring barley.

Soil tillage was performed before sowing each crop. In conventional tillage, moldboard ploughing was performed at a depth of 30 cm. Instead, in reduced tillage, chisel plowing was used at the same depth. Both systems were followed by disk harrowing at a depth of approximately 10 cm. For the herbaceous cash crops, phosphorus was applied before sowing as triple superphosphate (P2O5; 46%) at 109 kg ha^{-1}.

During GS1, winter wheat (Triticum aestivum L.; cv. RGT Bandera) was sown at a seeding rate of 250 kg ha-1. N fertilization was applied in two topdressing applications: urea (N; 46%) at 180 kg ha^{-1} at tillering, and ammonium nitrate (N; 26%) at 120 kg ha-1 at stem elongation, respectively. In GS2, according to the strip-plot arrangement, half of the plots were sown with red clover (Trifolium pratense L., cv. Rozeta) at a seeding rate of 20 kg ha^{-1}. The other half was sown with two-row spring barley (Hordeum vulgare L., cv. Fatima) at 225 kg ha^{-1}. N fertilization for the barley plots was applied as ammonium nitrate (N; 26%) at 193 kg ha^{-1} during tillering and 240 kg ha^{-1} at stem elongation.

In GS3, all plots were sown with two-row spring barley (cv. Fatima) at the same rate of 225 kg ha^{-1}, with N applied at the same timings and rates as reported for GS2. Throughout both seasons, no significant damage from pests or weeds was observed in the field.

TR (Raphanus sativus var. longipinnatus L.H. Bailey) was sown as a CC in both GS2 and GS3 at a seeding rate of 18 kg ha^{-1}. A spring tine harrow was used after TR sowing to enhance seed-to-soil contact. No fertilizers or pesticides were applied to the CC. CC termination was carried out by spreading glyphosate at 2 L ha^{-1}.

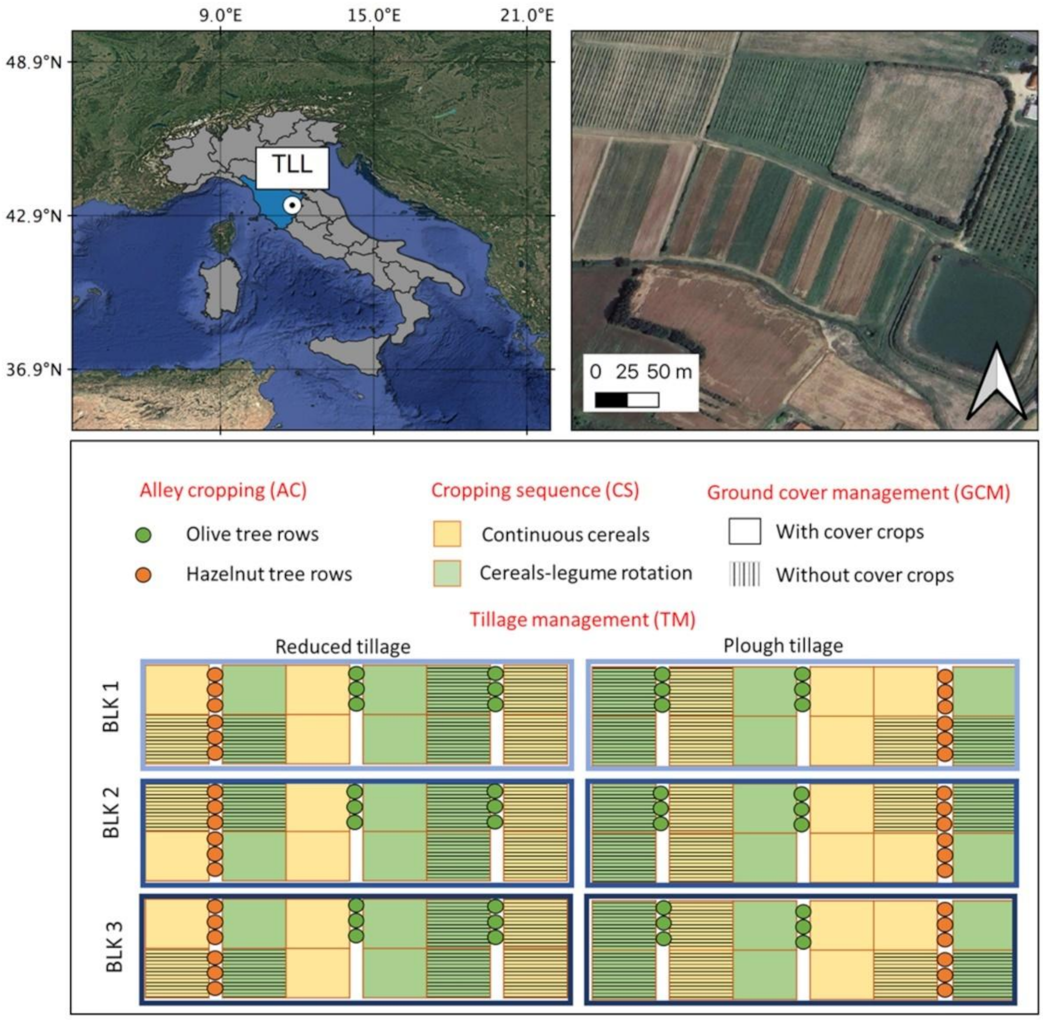

Figure 1 Localization of the Tereto Living Lab (TLL) and the correspondent experimental design with BLK stands for block

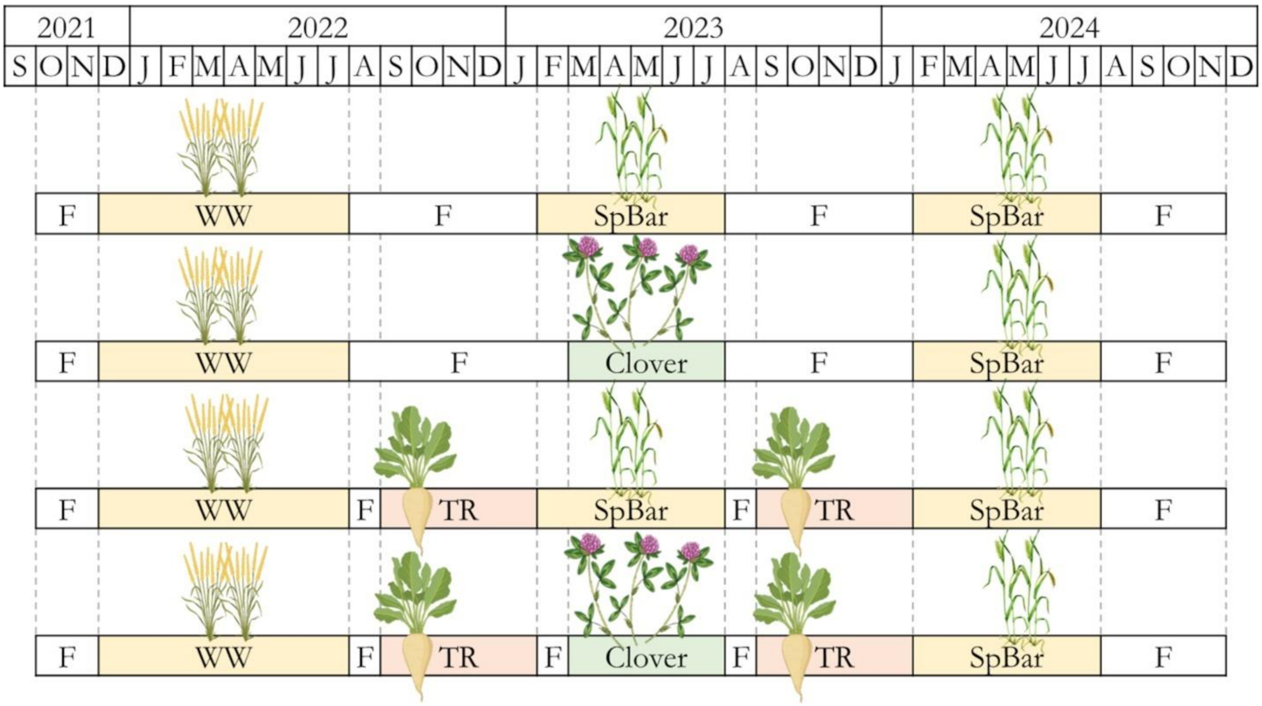

Figure S1 Schematization of the different treatments. Abbreviations are as follows: winter wheat (WW), spring barley (SpBar), tillage radish (TR), red clover (Clover), and fallow (F). The crop rotations were applied as monoculture and as olive-based and hazelnut-based alley cropping systems, respectively

7.3.4. Biomass and grain sampling

For each plot, three quadrats (50 cm x 50 cm) were harvested from the Border area (furrow, olive and hazelnut trees) and three quadrats from the Center area. The samples from the three quadrats in each area were combined to form a single, representative sample. This was performed by following standard composite sampling procedures, used to reduce within-plot variability and improve the reliability of soil and biomass analyses. Harvested biomass included cereal grain for cereals, as well as total aboveground biomass for both cereals and red clover. These biomass samples were considered as the harvestable product to be used for further analyses.

The total aboveground biomass (TBM) of both winter wheat and spring barley were harvested at commercial maturity (~<13% grain moisture), and then processed using a mechanical thresher to separate the straw yield (SY) from the grain yield (GY). Both the straw and grain fractions were subsequently ground separately using a Cyclotec 1093 lab mill (FOSS Tecator, Höganäs, Sweden) with a 0.5 mm screen (Guerrini et al. 2020) for laboratory analyses. Red clover biomass was oven-dried at 60 °C for 48 h until constant weight prior to analysis. Total nitrogen (TN) in red clover biomass, winter wheat and spring barley kernels was determined by combustion (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA). Crude protein content was calculated from the TN using conversion factors specific to each species as follows: 5.75 for winter wheat (ICC Standard 167, 2000), 5.45 for spring barley grain protein content (Feedipedia, 2025), and 6.25 for red clover biomass (Broderick, 2018).

7.3.5. Soil sampling and analysis

Soil samples were collected both before sowing and after harvesting each main crop—winter wheat, spring barley (during GS2 and GS3), and red clover—across all growing seasons. For each sampling event, three soil cores were taken at each of the three locations along both the Border and Center area, with cores at depths of 0–5 cm, 5–10 cm, and 10–20 cm (Sde), respectively, at each of the locations. Thus, a total of three soil cores were collected at each depth and position (Border and Center) for each alley cropping treatment (ACe) in each plot. These subsamples were combined to form a single representative sample for each plot-by-position-by-depth combination, according to standard composite sampling practices to reduce spatial variability and improve analytical reliability.

After collection, soil samples were air-dried and sieved to 2 mm, then analyzed for key soil-health indicators. Briefly, soil organic carbon (SOC) and TN were analyzed using a CHN analyzer (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA) after grinding the soil with a planetary ball mill. Particulate organic carbon (POM-C) and permanganate-oxidizable carbon (POX-C) were measured according to the methods of Cambardella and Elliott (1992) and Weil et al. (2003), respectively. Microbial biomass carbon (MBC), phosphorus (MBP), and nitrogen (MBN) were determined using the fumigation-extraction method (Vance et al. 1987; Brookes et al. 1985). Extracts were analyzed using a CHN analyzer for MBC and MBN, while MBP was determined colorimetrically. Microbial biomass values were adjusted using established correction coefficients as follows: 0.38 for MBC, 0.40 for MBP, and 0.54 for MBN (Joergensen, 1996; Brookes et al., 1982; Joergensen & Müller, 1996).

7.3.6. Pre-crop level – effects on grain, biomass, and protein yield

The spring barley harvested during GS1 and GS2 was analyzed on the pre-crop level, following the approach of Simon-Miquel et al. (2024). Specifically, we evaluated the effect of the preceding crop, either nCC or CC using TR, on spring barley performance across both GSs. This analysis focused on TR as the pre-crop because it causes minimal disruption to the cereal monocropping calendar, requiring only sowing and, where necessary, termination without altering the main crop cycle. Thus, farmers could adopt this practice without significantly modifying their conventional systems. In addition, tillage radish has been associated with increased available phosphorus in the soil, a key benefit in the TLL where available P was low. We also accounted for the effect of tillage management and growing season, as these factors could differently affect the performances of spring barley.

7.3.7. Cropping system level – effects on energy, biomass, and protein yield

The overarching productivity of the cropping systems was evaluated based on energy yield (EY), protein yield (PY), and the TBM of the main herbaceous crops (winter wheat, spring barley, red clover). EY (GJ ha^{-1}) was calculated by multiplying GY by specific gross energy conversion factors. Specifically, the conversion factors for winter wheat and spring barley were 18.2 and 18.4 MJ kg^{-1}, respectively (Simon-Miquel et al., 2024). The conversion factor for fresh red clover was calculated multiplying the total TBM by the conversion factor 18.4 MJ kg^{-1} (Feedipedia, 2025). TBM yield of herbaceous crops was compared across cropping systems to capture productivity differences. To evaluate nitrogen efficiency, both PY and EY were divided by the total synthetic nitrogen fertilizer content applied over all growing season according to the following equations:

$$NUE_{synt-e} = \frac{\sum energy\ yield\ (GJ\ ha^{-1})}{\sum synthetic\ N\ fertilizer\ applied\ (\ kg\ ha^{-1})}$$

$$NUE_{synt-p} = \frac{\sum protein\ yield\ (kg\ ha^{-1})}{\sum synthetic\ N\ fertilizer\ applied\ (\ kg\ ha^{-1})}$$

Where $NUE_{synt\text{-}e}$ and $NUE_{synt\text{-}p}$ represent nitrogen use efficiency for energy and protein yield, respectively. These metrics, based on Simon Miquel et al. (2023), permit the assessment of the capacity of the system to convert applied N into usable energy and protein outputs.

7.3.8. Statistical analysis

For each of the following models, the assumptions of normality and homoscedasticity were assessed using the Shapiro-Wilk test and Levene's test, respectively. When data did not meet the mentioned assumptions, a Box-Cox transformation was applied.

The analyses were performed both on the pre-crop level and on the cropping system levels as reported in Simon-Miquel et al. (2024). The following mixed model was used to evaluate the pre-crop effect of GCM, TM, and GS (fixed factors) on the performances of spring barley:

$$Y_{ijk} = \beta_0 + \sum_{i=1}^{3} \beta_i\, x_i + \sum_{i<j} \beta_{ij}\, x_i x_j + \beta_{123} x_1 x_2 x_3 + blk + \epsilon$$

Where Yijk is the grand mean of the considered parameter (GY, SY, and PY of spring barley), β_0 represents the intercept; x_1, x_2, and x_3 are the main factors, corresponding to GS, TM, and GCM; $\beta_i x_i$ is the effect of the main factors for each individual factor (GS, TM, and GCM); $\beta_{ij} x_i x_j$ is the effect of the two-way interactions; $\beta_{123} x_1 x_2 x_3$ represents the three-way interaction; blk is the block factor, which was considered random.

The analysis of cropping system performance was conducted by summing the values recorded for each system across the three growing seasons. Therefore, the results refer to the cumulative performance over a three-year period. A mixed model was used for evaluating the effect of the different practices on the EY, PY, TBM, NUE_{synt-e}, and NUE_{synt-p} of the cropping system, respectively. The model is reported below:

$$Y_{ijk} = \beta_0 + \sum_{i=1}^{4} \beta_i\, x_i + \sum_{i<j} \beta_{ij}\, x_i\, x_j + \sum_{i<j<k} \beta_{ijk}\, x_i x_j x_k + \beta_{1234}\, x_1 x_2 x_3 x_4 + blk + \epsilon$$

where Y_{ijk} is the grand mean of the considered cropping system parameter; x_1, x_2, x_3, and x_4 are the main fixed factors, corresponding to TM, CS, GCM, and AC, respectively; $\beta_i x_i$ is the main effect of the main factor i; $\beta_{ij} x_i x_j$ is the two-way interaction between factors x_i and x_j; $\beta_{ijk} x_i x_j x_k$ is the three way interaction, and $\beta_{1234} x_1 x_2 x_3 x_4$ represents the four-way interaction between all factors.

As regards the soil parameters, the data collected at the end of each GSs were subtracted from the initial values just before the sowing of the cash crop for each GS. Therefore, the soil data are expressed in terms of differentials (Δ) between post- and pre-sowing values for each GS. A mixed model was used for evaluating the effect of the different practices on soil properties:

$$Y_{ijk} = \beta_0 + \sum_{i=1}^{6} \beta_i x_i + \sum_{i<j} \beta_{ij} x_i x_j + \sum_{i<j<k} \beta_{ijk} x_i x_j x_k + \sum_{i<j<k<l} \beta_{ijkl} x_i x_j x_k x_l + \sum_{i<j<k<l<m} \beta_{ijklm} x_i x_j x_k x_l x_m + \beta_{123456}\, x_1 x_2 x_3 x_4 x_5 x_6 + blk + \epsilon$$

where Y_{ijk} is the grand mean of the considered soil; x_1, x_2, x_3, x_4, x_5, and x_6 are the main factors, corresponding to TM, CS, GCM, AC, ACe, Sde, respectively; $\beta_i x_i$ is the main effect of the main fixed factor i; $\beta_{ij} x_i x_j$ is the two-way interaction between factors x_i and x_j; $\beta_{ijk} x_i x_j x_k$ is the three way interaction; $\beta_{ijkl} x_i x_j x_k x_l$ is the three way interaction; $\beta_{ijklm} x_i x_j x_k x_l x_m$ is the three way interaction, and $\beta_{123456} x_1 x_2 x_3 x_4 x_5 x_6$ represents the six-way interaction between all factors.

For all the models mentioned, when ANOVA yielded significant results, a post hoc Tukey's test ($p < 0.05$) was conducted to assess pairwise differences between treatments.

7.4. Results

7.4.1. Climate description

Over the duration of the growing seasons 1 (GS1; 2021/2022), 2 (GS2; 2022/2023), and 3 (GS3, 2023/2024), the TLL experienced notable deviations in rainfall and temperature compared to the long-term averages (1980-2020) (Figure 2). In particular, in the March–June period, rainfall was 115 mm (GS1), 301 mm (GS2), and 280 mm (GS3). The long-term average rainfall was approximately 262 mm. Meanwhile, the average temperatures during the key period (April–July) were consistently higher across all three seasons than the historic norm. Compared to the long-term average of about 14 °C, GS2 and GS3 recorded means of 14.7 °C and 14.8 °C, respectively. GS1 was shown to average around 15 °C between March and June. These deviations suggested that GS2 and GS3 were characterized by wetter and warmer spring conditions than usual, while GS1 was notably drier.

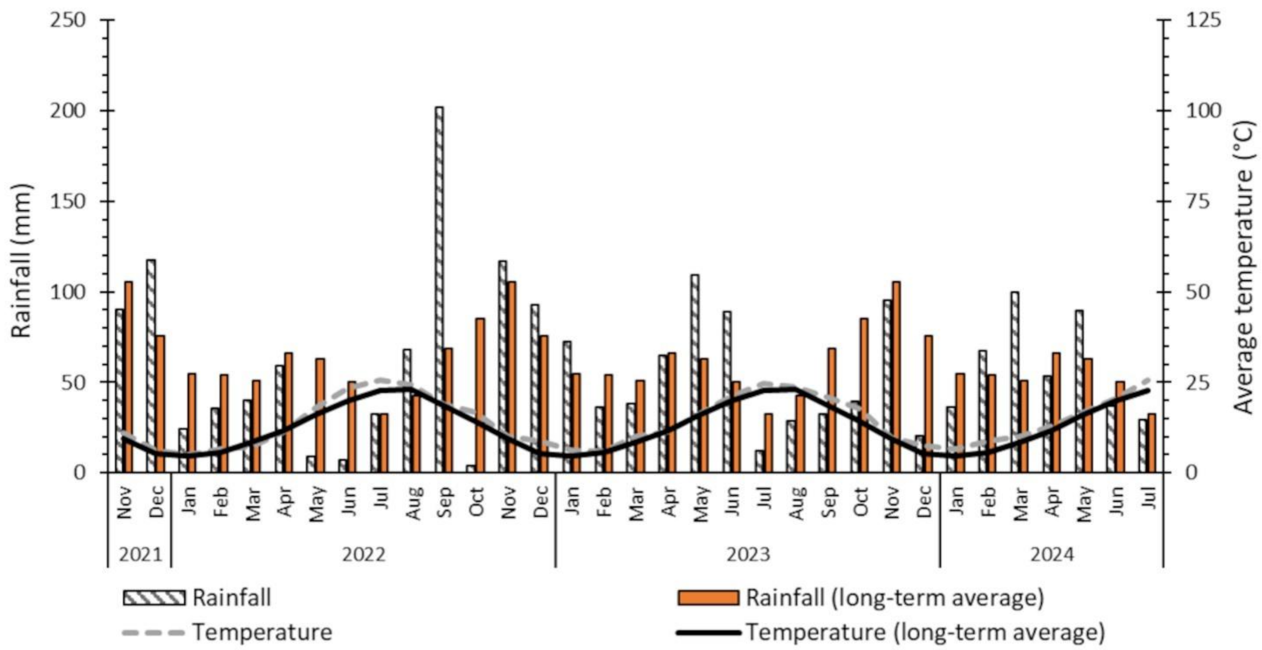

Figure 2 Walter-Lieth diagram for the Tereto Living Lab (TLL Marciano della Chiana, Arezzo, Italy) during the experimental period, with long-term averages of rainfall and temperature from 1980 to 2020.

7.4.2. Pre-crop effect- Spring Barley performances

Spring barley performances were significantly influenced by the combined effects of pre-crop ground cover management (GCM), tillage management (TM), and growing season (GS), as shown in Table 2. Among these, GCM had a notable impact on grain yield (GY). Plots where barley followed a cover crop (CC), specifically tillage radish (TR), there was an average GY of 3.68 t ha^{-1}. This value was approximately 27% higher than the yield observed in nCC plots, where barley followed fallow (Table 3). A significant interaction was also observed between TM x GS. During GS2, the grain yield measured under ploughing (Plough) was significantly higher by 71.4% compared to reduced (Red) tillage (Figure 3). In GS3, the difference between tillage treatments was still significant, but less pronounced, with a 34.9% higher GY under Plough than under Red. Moreover, within the TM treatments, the GY in GS2 was significantly higher by approximately 24% than in GS3 (Figure 3).

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Table 2 Anova table for the pre-crop effect of ground cover management (GCM), tillage management (TM), and growing season (GS) on the performances of spring barley. Significant differences are indicated by *** (p < 0.001), ** (p < 0.01), * (p < 0.05), and "ns" (not significant)

Variability sources	GY		SY		PY	
	F value	sig	F value	sig	F value	sig
GCM	45.13	***	0.08	ns	39.14	***
TM	156.00	***	28.49	***	130.13	***
GS	12.35	***	145.67	***	7.11	**
GCM × TM	0.07	ns	6.13	*	0.05	ns
GCM × GS	0.77	ns	7.24	**	2.15	ns
TM × GS	17.19	***	0	ns	17.9	***
GCM × TM × GS	0.7	ns	0.7	ns	1.69	ns

GY= grain yield; SY= straw yield; PY= protein yield

Table 3 Mean values (± standard error) of spring barley grain yield (GY), straw yield (SY), and protein yield (PY) across treatments. Means followed by different lowercase letters within each factor are significantly different (Tukey's HSD,

$p < 0.05$). GCM: comparison between plots with cover crop (CC, preceded by tillage radish) and without cover crop (nCC, preceded by fallow); TM: tillage management, with reduced tillage vs. plough tillage (Red vs Plough); GS: growing season, comparing GS2 and GS3.

Variability sources	Levels	GY		SY		PY	
		t ha^{-1}	sig	t ha^{-1}	sig	kg ha^{-1}	sig
GCM	CC	3.68 (0.10)	a	4.54 (0.16)		418.4 (12.77)	a
	nCC	2.90 (0.09)	b	4.32 (0.18)		321.07 (11.86)	b
TM	Red	2.67 (0.08) b	b	3.94 (0.17)	b	297.61 (10.21)	b
	Plough	3.92 (0.10) a	a	4.91 (0.15)	a	441.86 (12.11)	a
GS	GS2	3.55 (0.14)	a	5.90 (0.21)	a	395.38 (16.69)	a
	GS3	3.16 (0.09)	b	3.69 (0.10)	b	356.91 (11.08)	b

Figure 3 Effect of the interaction of tillage management and growing season (TM × GS) on grain yield (GY) of spring barley. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). Lowercase letters indicate significant differences between the TM (conventional [Plough] vs reduced tillage [Red]) within the GS. Error bars indicate the standard error. Uppercase letters indicate significant differences between GS (2^{nd} growing season vs 3^{rd} growing season) within the TM.

The straw yield (SY) of spring barley was significantly influenced by TM ($p < 0.001$), GS ($p < 0.001$), and their interactions with GCM (Table 2). Although the main effect of GCM was not significant, a significant GCM × TM interaction ($p < 0.05$) revealed that tillage effects varied depending on the presence of cover crops (Table 2). Specifically, plots with a preceding CC produced significantly higher straw yields, by 29.2%, compared to those under nCC (Figure 4). Moreover, within the CC treatment, the SY under Plough was significantly higher by 35.6% than under Red. Conversely, in the absence of a cover crop, the difference between the tillage treatments was not statistically significant. The interaction GS × GCM also had a significant effect on SY ($p < 0.01$). Overall, the SYs recorded were consistently higher in GS2 than in GS3, with increases of 43.3% and 78.9% for CC and nCC treatments, respectively. During GS3, the SY of spring barley measured in CC plots remained significantly higher by about 16% than that in nCC. This indicated a residual benefit of the cover crop under less favorable seasonal conditions.

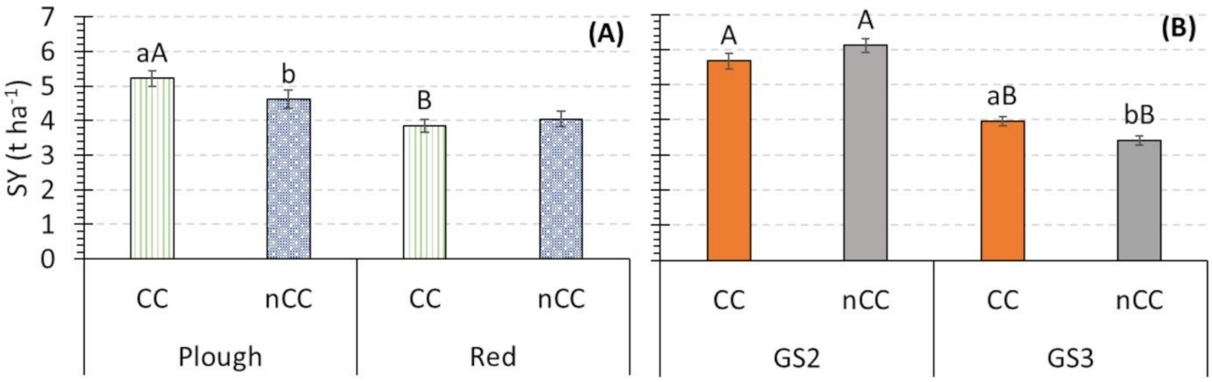

Figure 4 Effect of the interaction of (A) tillage management and ground cover management (TM × GCM) and (B) growing season and ground cover management (GS × GCM) on straw yield (SY) of spring barley. Error bars represent standard errors. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). In TM × GCM, lowercase letters indicate significant differences between GCM (cover crops [CC] vs. no cover crop [nCC]), within TM, while uppercase letters indicate significant differences between TM (conventional [Plough] vs reduced tillage [Red]) within GCM. In GS × GCM, lowercase letters indicate significant differences between GCM within GS, while uppercase letters indicate differences between GS (2nd growing season vs 3rd growing season) within GCM.

The protein yield (PY) of spring barley was significantly affected by GCM ($p < 0.001$), TM ($p < 0.001$), and GS ($p < 0.01$) (Table 2). Plots in which spring barley followed CC were shown to attain an average PY, significantly higher by 30.3% compared to that measured in nCC (Table 3). TM had a strong main effect, with PY under Plough outperforming the PY measured under Red. A significant interaction between GS × TM ($p < 0.001$) was also observed (Figure 5). In GS2, PY under Plough was approximately double that recorded under Red. Although this difference was less pronounced in GS3, Plough still produced 34% higher PY than Red. Within Plough, PY in GS2 was significantly greater by 24.4%, than in GS3, suggesting seasonal variability played a key role in modulating treatment effects.

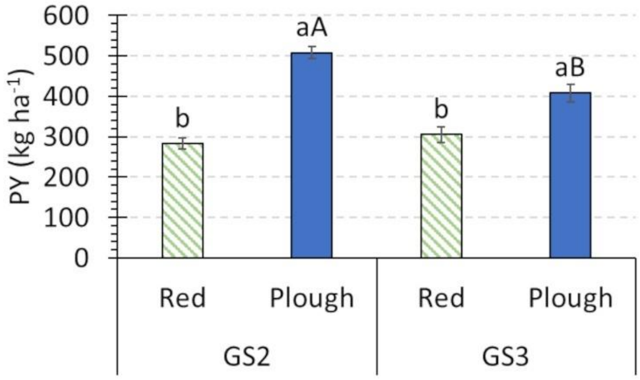

Figure 5 Effect of the interaction between tillage and growing season (TM × GS) on protein yield (PY) of spring barley. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). Lowercase letters indicate significant differences between TM (conventional [Plough] vs reduced tillage [Red]) within GS. Error bars indicate the standard error. Uppercase letters indicate significant differences between GS (2nd growing season vs 3rd growing season) within TM.

7.4.3. Cropping systems performances

Energy yield (EY) was significantly affected by all main factors, TM, cropping sequence (CS), GCM, and alley cropping configuration (AC), as well as by selected two-way interactions, as indicated by the ANOVA results (Table 4). Among the main effects, Plough increased EY by 34.4% compared to reduced tillage. Of the CS, crop rotation (ROT) outperformed continuous cereal (CONTIN) system by 6.7%, and CC exceeded nCC by 9.3% (Table 5). The EY was significantly influenced by the interaction between TM × CS (Table 4). The TM × CS interaction showed that, under Plough, EY increased by 43.8% in CONTIN and 26.3% in ROT relative to Red (Figure 6A). Instead, ROT under Red conferred a 15.2% advantage over CONTIN. The interaction TM × AC also had a significant effect on EY (Table 4). Across all AC, Plough resulted in higher EY values compared to Red (Figure 6B). EY under Plough increased by 61% in hazelnut-based alley cropping (Haz), 33% in monoculture (Mono), and 27% in olive-based alley cropping (Oliv), compared to Red. Under Red, the Oliv and Mono systems outperformed the Haz by 30–32%. The GCM × CS interaction showed that CC enhanced EY by 20.2%

in the Mono and 24.7% in the Oliv relative to the Haz, with an additional 17.6% gain observed within the Oliv compared to nCC conditions.

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Table 4: Anova table for the influence of different factors on the performances of the cropping system. Significant differences are indicated by *** (p < 0.001), ** (p < 0.01), * (p < 0.05), and "ns" (not significant)

Variability source	EY		PY		TBM		NUE_{synt-e}		NUE_{synt-p}	
	F value	sig	F value	sig	F value	sig	F value	sig	F value	sig
TM	210.70	***	164.01	***	225.62	***	200.98	***	134.8467	***
CS	5.91	*	111.62	***	46.86	***	431.56	***	575.4867	***
GCM	13.06	***	5.47	*	4.68	*	12.43	***	4.9836	*
AC	14.97	***	17.27	***	7.45	***	16.47	***	17.589	***
TM x CS	6.52	*	1.62	ns	6.37	*	0.13	ns	1.4248	ns
TM x GCM	1.53	ns	0.00	ns	5.54	*	1.48	ns	0.0052	ns
CS x GCM	0.42	ns	0.14	ns	0.02	ns	0.01	ns	0.6053	ns
TM x AC	4.19	*	1.36	ns	10.36	***	4.22	*	1.1277	ns
CS x AC	2.25	ns	4.27	*	4.67	*	3.96	*	6.9098	**
GCM x AC	4.45	*	2.58	ns	4.19	*	3.09	ns	2.0002	ns
TM x CS x GCM	0.03	ns	0.12	ns	0.48	ns	0.00	ns	0.1061	ns
TM x CS x AC	0.03	ns	0.17	ns	0.72	ns	0.13	ns	0.1531	ns
TM x GCM x AC	0.22	ns	0.07	ns	0.16	ns	0.18	ns	0.0805	ns
CS x GCM x AC	3.60	ns	1.05	ns	4.45	ns	2.26	ns	0.7404	ns
TM x CS x GCM x AC	0.37	ns	0.32	ns	0.76	ns	0.32	ns	0.2856	ns

EY= energy yield; PY= protein yield; TBM= total biomass; NUE_{synt-e}= synthetic nitrogen use efficiency for energy yield; NUE_{synt-p} = synthetic nitrogen use efficiency for protein yield; TM= Tillage management; CS= cropping sequence; GCM=ground cover management; AC= alley cropping configuration

Table 5: Effects of the main factors on the performance of the cropping system over the three-year trial period. Results are expressed as the means with standard errors (in parenthesis). Different letters denote statistically significant differences among the levels of each factor.

Variability source		EY (GJ ha^{-1})	PY (kg ha^{-1})	TBM (t ha^{-1})	NUE_{synt-e} (GJ kg N^{-1})	NUE_{synt-p} (kg prot kg N^{-1})
TM	plough	257.29 (3.48) a	1869.58 (39.88) a	31.47 (0.47) a	0.98 (0.03) a	7.25 (0.30) a
	reduced	191.46 (4.31) b	1365.97 (44.23) b	23.67 (0.54) b	0.74 (0.03) b	5.37 (0.27) b
CS	continuous	217.10 (6.12) b	1376.44 (40.10) b	29.51 (0.76) a	0.66 (0.02) b	4.17 (0.12) b
	rotation	231.65 (4.73) a	1859.11 (45.66) a	25.62 (0.51) b	1.05 (0.02) a	8.45 (0.21) a
GCM	CC	234.39 (5.85) a	1677.13 (53.28) a	28.14 (0.75) a	0.89 (0.03) a	6.55 (0.32) a
	nCC	214.36 (4.91) b	1558.43 (48.95) b	26.99 (0.60) b	0.82 (0.03) b	6.08 (0.29) b
AC	mono	230.64 (5.26) a	1660.90 (52.81) a	27.89 (0.63) a	0.88 (0.03) a	6.50 (0.32) a
	hazelnut	197.70 (10.82) b	1375.25 (80.09) b	25.35 (1.46) b	0.75 (0.05) b	5.31 (0.42) b
	olive	228.31 (6.19) a	1674.36 (58.68) a	28.20 (0.80) a	0.87 (0.03) a	6.52 (0.37) a

EY= energy yield; PY= protein yield; TBM= total biomass; NUEsynt-e= synthetic nitrogen use efficiency for energy yield; NUEsynt-p = synthetic nitrogen use efficiency for protein yield; TM= tillage management (conventional [Plough] vs reduced tillage [Red]); CS= cropping sequence (Continuous cereal cropping [CONTIN] vs Cereal-legume rotation [ROT]); GCM=ground cover management (cover crops [CC] vs. no cover crop [nCC]); AC= alley cropping configuration (hazelnut-based [Haz] and olive-based [Oliv] alley cropping systems and herbaceous monoculture [Mono])

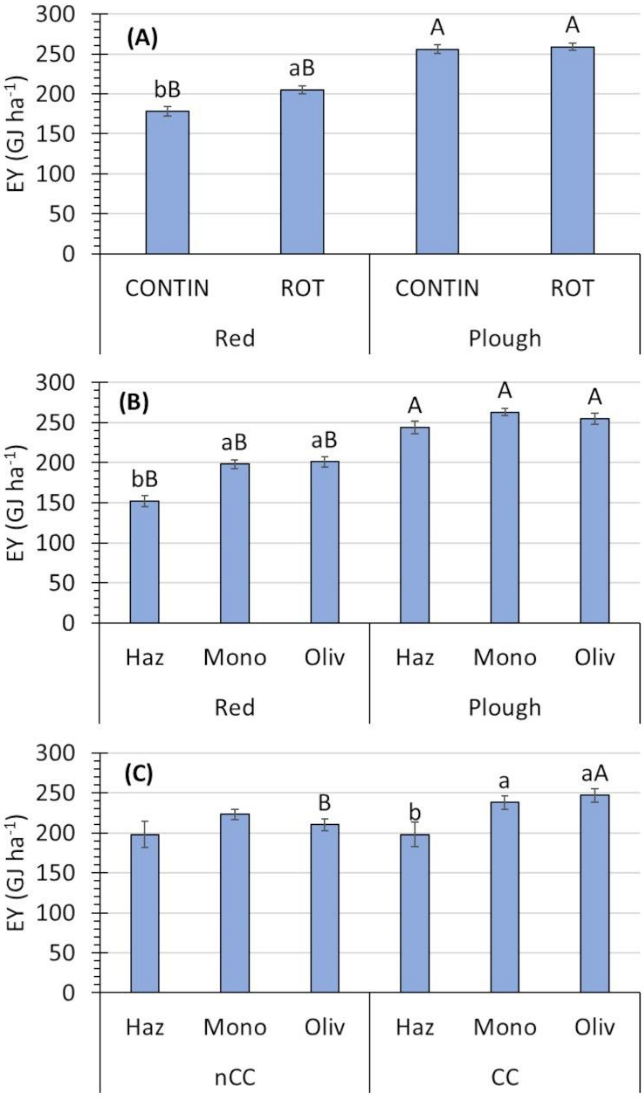

Figure 6: Effect of the interaction between **(A)** tillage management and cropping sequence (TM × CS), **(B)** tillage management and alley cropping configuration (TM x AC), and **(C)** ground cover management and alley cropping configuration (GCM × AC) on the energy yield (EY) of the system over the three-year period. Results are expressed as the means with standard errors. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). In TM × CS, lowercase letters indicate differences between CS (Continuous cereal cropping [CONTIN] vs Cereal-legume rotation [ROT]), while uppercase letters report differences between TM (reduced [Red] vs conventional [Plough] tillage). In TM × AC, lowercase letters indicate differences among AC (hazelnut-based [Haz] and olive-based [Oliv] alley cropping systems and herbaceous monoculture [Mono]), while uppercase letters indicate differences between TM. In GCM × AC, lowercase letters indicate differences between AC while uppercase letters report differences between GCM (cover crops [CC] against no cover crop [nCC]).

The PY was significantly affected by TM ($p < 0.001$), CS ($p < 0.001$), GCM ($p < 0.05$), and AC ($p < 0.001$), and by the interaction between CS × AC (Table 4). Plough resulted in a 37% higher average PY than that measured under Red (Table 5), while CONTIN-to-ROT cropping increased PY by 35%. The introduction of CC significantly increased PY by 7.6% compared to nCC. The interaction between CS and AC also influenced the PY of the system (Table 4). Specifically, under AC, Mono and Oliv yielded approximately 41% and 33% more PY, respectively, than the CONTIN (Figure 7). Within the rotation treatment, both Mono and Oliv achieved significantly higher PY than the Haze alley system, with increases of 29.2% and 27.1%, respectively.

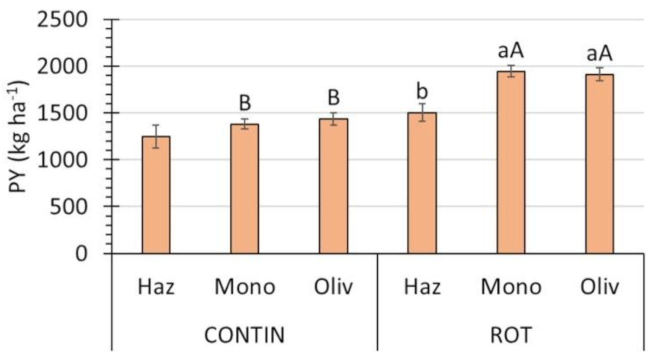

Figure 7: Effect of the interaction between cropping sequence and ally cropping configuration (CS × AC) on the protein yield (PY) over the three-year period. Results are expressed as the means with standard errors. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). Lowercase letters indicate differences between AC (hazelnut-based [Haz] and olive-based [Oliv] alley cropping systems and herbaceous monoculture [Mono]), while uppercase letters report differences between CS (Continuous cereal cropping [CONTIN] against Cereal-legume rotation [ROT]).

The ANOVA revealed significant effects of TM ($p < 0.001$), CS ($p < 0.001$), GCM ($p < 0.05$), and AC ($p < 0.001$), respectively, on total aboveground biomass yield (TBM) (Table 4). There were also significant effects between the interactions: TM × CS, TM × GCM, TM × AC, CS × AC, and GCM × AC (Table 4). Under Plough, TBM increased by approximately 33% relative to reduced tillage (31.5 vs 23.7 t ha^{-1}) (Table 5). The TM × CS interaction showed that, for CONTIN, conventional tillage increased TBM by 36%, and for ROT by 29% compared to reduced tillage (Figure 8C).

The TM × AC interaction revealed that the Haz displayed the largest TBM increase under Plough (67%), while Mono and Oliv increased by approximately 32% and 22%, respectively (Figure 8A). Within reduced tillage, TBM in Haz was 24.3% and 18.1% lower than in Mono and Oliv, respectively (Figure 8A). The TBM of the system was also significantly influenced by the interaction TM x GCM (Figure 8B). For the TM × GCM, Plough improved TBM by 39.6% and 27% with CC and nCC, respectively. Additionally, under plough tillage, CC resulted in a 9% gain in TBM compared to nCC. Finally, under Plough, CONTIN produced 18% more biomass than ROT, whereas under Red the increase was 11.6% in favor of CONTIN.

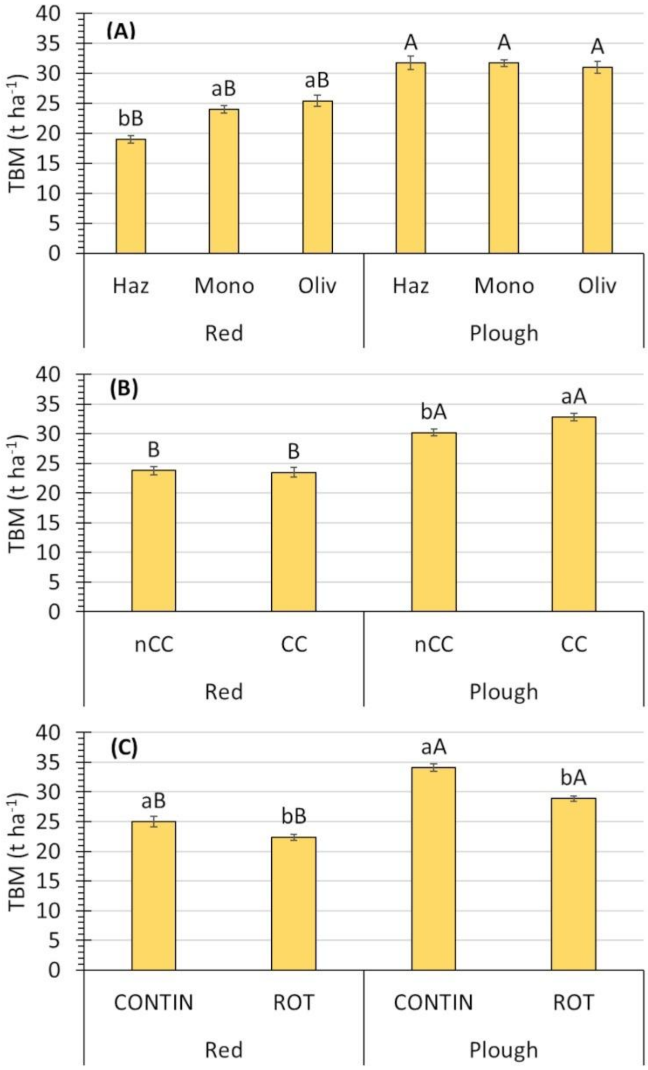

Figure 8: Effect of the interaction between **(A)** tillage management and alley cropping configuration (TM × AC), **(B)** tillage management and ground cover management (TM × GCM), and **(C)** tillage management and cropping sequence (TM × CS) on the total biomass yield (TBM) of each system (excluding the cover crop biomass) over the three-year period. Results are expressed as the means with standard errors. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). In TM × AC, lowercase letters indicate differences between AC (hazelnut-based [Haz] and olive-based [Oliv] alley cropping systems and herbaceous monoculture [Mono]), while uppercase letters report differences between TM (reduced [Red] vs conventional [Plough] tillage). In TM × GCM, lowercase letters indicate differences between GCM (cover crops [CC] vs no cover crops [nCC]), while uppercase letters report differences between TM. In TM × CS, lowercase letters indicate differences between CS (Continuous cereal

cropping [CONTIN] against Cereal-legume rotation [ROT]). Uppercase letters report differences between TM, while lowercase letters indicate differences between CS.

The ANOVA showed that NUE_{synt-e} was significantly influenced by TM, CS, GCM and AC, ($p < 0.001$ for all), with significant interactions detected between TM × AC and CS × AC (Table 4). Plough tillage increased NUE_{synt-e} by 32% compared to Red. ROT improved NUE_{synt-e} by 59% over CONTIN, and CC enhanced NUE_{synt-e} by 8.5% relative to nCC (Table 5). Under ROT, NUE_{synt-e} increased by 67%, 52%, and 54% in Mono, Oliv, and Haze alley systems, respectively, compared to their CONTIN counterparts. Mono was shown to outperform Haz by 21% (Figure 9A). Plough boosted NUE_{synt-e} by 57%, 32%, and 25% in Haz, Mono and Oliv alley systems, respectively, compared to Red (Figure 9B).

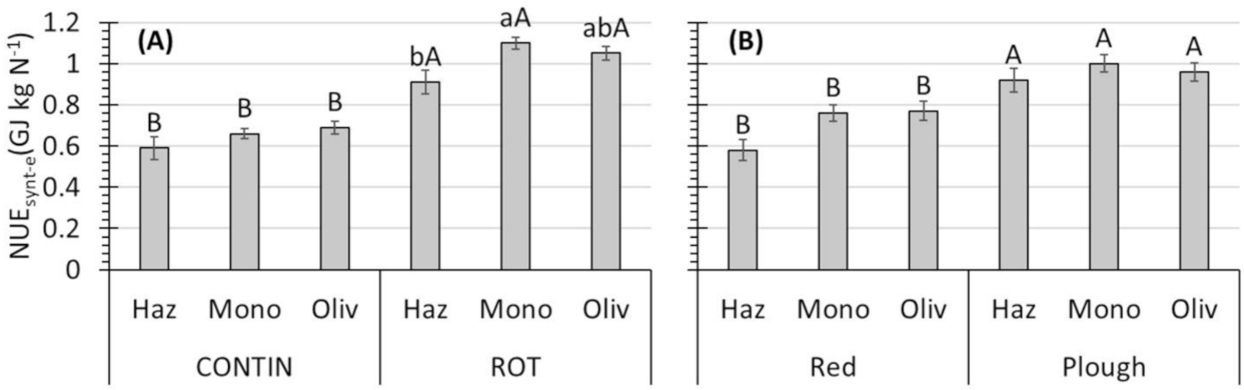

Figure 9: Effect of the interaction between **(A)** cropping sequence and alley cropping configuration (CS × AC), and **(B)** tillage management and alley cropping configuration (TM × AC) on the nitrogen use efficiency in terms of energy yield (NUE_{synt-e}) over the three-year period. Results are expressed as the means with standard errors. Different letters denote statistically significant differences among treatments based on Tukey's HSD post-hoc test ($p < 0.05$). In CS × AC, lowercase letters indicate differences among AC (hazelnut-based [Haz] and olive-based [Oliv] systems and herbaceous monoculture [Mono]), while uppercase letters report differences between CS (Continuous cereal cropping [CONTIN] against Cereal-legume rotation [ROT]). In TM × CS, lowercase letters indicate differences between AC, while uppercase letters indicate differences between TM (reduced [Red] vs conventional [Plough] tillage).

The ANOVA demonstrated that NUE_{synt-p} was significantly affected by TM ($p < 0.001$), CS ($p < 0.001$), GCM ($p < 0.05$) and AC ($p < 0.001$), as well as by the CS × AC (Table 4). In general, a significantly higher NUE_{synt-p} was observed under Plough and in the presence of CC (Table 5). Plough increased NUE_{synt-p} by 35% compared to Red, while CC resulted in a 7.8% increase relative to nCC (Table 5). ROT nearly doubled NUE_{synt-p} compared to CONTIN (Figure 10). Mono and Oliv alley systems exhibited significantly higher NUE_{synt-p} than the Haze alley by 29.1% and 27.0%, respectively.

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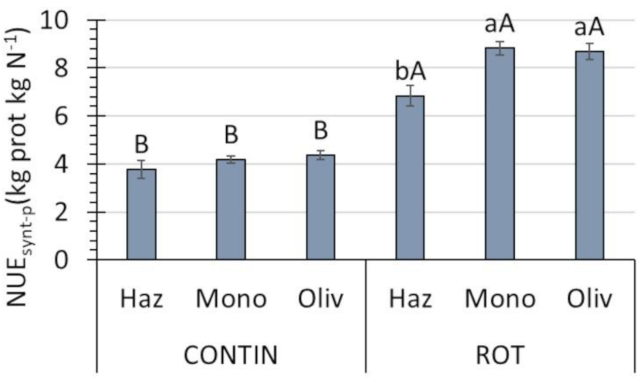

Figure 10: Effect of the interaction between cropping sequence and alley cropping configuration (CS × AC) on the nitrogen use efficiency in terms of protein yield (NUE_{synt-p}) over the three-year period. Results are expressed as the means with standard errors. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). Lowercase letters indicate differences among AC (hazelnut-based [Haz] and olive-based [Oliv] systems and herbaceous monoculture [Mono]), while uppercase letters report differences between CS (Continuous cereal cropping [CONTIN] against Cereal-legume rotation [ROT]).

7.4.4. Soil response to the adoption of different sustainable practices

Over the 3-year period, changes in soil parameters were calculated as the difference between post- and pre-main crop soil samples. Consequently, the analyses were based on the differences between pre- and post-main crop values, expressed as differentials (Δ). In particular, Plough significantly increased ΔSOC by 52.9% compared to Red (Table 6), and CC led to a 68.7% higher ΔSOC compared to nCC. AC treatments (Mono, Haze and Oliv) did not significantly affect ΔSOC, nor did sample position within the plot. SOC values decreased slightly with increasing depth. ΔPOM-C displayed significant responses to TM ($p < 0.05$), GCM (GCM) ($p < 0.01$), and the three-way interaction TM × GCM × AC ($p < 0.05$) (Table S3). Positive ΔPOM-C values were recorded under Plough and in presence of CC, whereas Red resulted in negligible or negative changes (Table 6). Likewise, CC treatments increased ΔPOM-C, compared with slightly negative values under nCC conditions. Effects of CS, AC, and Sde were non-significant. These results highlighted that mechanical disturbance combined with cover cropping promoted accumulation of labile organic carbon fractions. POX-C generally declined across treatments, except at the 10-20 cm soil depth, where ΔPOX-C values significantly increased compared to the upper layers. Statistical analysis revealed that soil depth and the interaction GCM × AC were the only highly significant factors ($p < 0.001$), while main effects of TM and CS showed nonsignificant but noticeable trends (Table S2). Mean ΔPOX-C values were negative in the topsoil (0–5 cm: –8.1 mg kg^{-1}; 5–10 cm: –13.1 mg kg^{-1}), indicating overall depletion (Table 6). By contrast, the 10–20 cm layer exhibited a positive mean change of +2.9 mg kg^{-1}. All AC systems exhibit negative ΔPOX-C values (Figure 11), indicating depletion, except for the Mono with nCC, which shows a slight positive change. The greatest decreases are observed in Haz and Mono systems with CCs. The ΔMBC was significantly affected by TM, CS, and GCM (Table S2). Reduced tillage and the adoption of CCs both resulted in higher ΔMBC compared to plough tillage and nCC. A significant CS × GCM interaction revealed that under Red with CCs, ΔMBC was more than double than that observed under nCC—regardless of rotation (Figure 12). Specifically, in CONTIN, ΔMBC under CC management was 84.3% greater than in the nCC. Additionally, ΔMBC declined with soil depth, particularly below 10 cm (Table 5).

Table S2: Analysis of variance (ANOVA) showing the effects of different factors on key soil properties over the three-year period. Significance levels are indicated as: *** (p < 0.001), ** (p < 0.01), * (p < 0.05), and "ns" (not significant)

Source of variability	Df	ΔSOC		ΔPOM-C		ΔPOX-C		ΔMBC		ΔOlsP		ΔMBP		ΔNtot		ΔMBN	
		Sum Sq	sig	Sum Sq	sig	Sum Sq	sig	Sum Sq	sig	Sum Sq	sig	Sum Sq	sig	Sum Sq	sig	Sum Sq	sig
TM	1	2.78	**	0.32	*	1162		84256	***	3.57	*	79.95	***	13611		774	***
CS	1	0.38		0.02		336		26428	*	0.42		43.16	***	13482		598.6	**
GCM	1	3.78	***	0.6	**	184		240018	***	4.46	*	168.49	***	1111		2014.4	***
AC	2	0.04		0.12		1110		8042		0.8		2.68		9438		13.4	
Ace	1	0.18		0.01		701		1211		0.28		0.7		2904		2.5	
Sde	2	0.2		0.3		57664	***	652772	***	23.98	***	230.72	***	2580		1819.6	***
TM × CS	1	0		0.08		244		16828		0.5		29.61	**	1470		277.2	*
TM × GCM	1	0.02		0		892		1494		0.6		2.29		1427		18	
CS × GCM	1	0.11		0.16		1434		22283	*	1.37		8.14		10112		43.3	
TM × AC	2	0		0.08		1780		52980		0.14		45.86	**	2672		281.2	
CS × AC	2	0.1		0.22		218		13588		1.62		4.92		862		174.6	
GCM × AC	2	0.02		0.26		16664	***	26872		0.4		1.1		4390		111	
TM × ACe	1	0.05		0.02		430		30		0		0.02		378		1.3	
CS × ACe	1	0.15		0.02		628		579		0.11		0.42		598		0.8	
GCM × ACe	1	0.25		0.06		139		201		0.05		0.73		1736		1	
AC × Ace	2	0.02		0.04		1020		528		2.58		0.94		4652		16	

TM × Sde	2	0.02	0.06	2496	23438	2.8	2.08	222	87.2
CS × Sde	2	0.1	0.1	1442	18444	0.92	9.42	7310	17.4
GCM × Sde	2	0.2	0.38	1030	53688	0.62	8.82	3614	331.6
AC × Sde	4	0.2	0.04	2908	5912	5.96	4.36	4372	91.2
ACe × Sde	2	0.34	0.1	132	256	0.86	2.62	2352	1.6
TM × GCM × AC	2	0.02	0.44 *	2622	93182 **	0.02	41.76 **	104	574 **
Other 3-way interactions	42	2.93	2.99	36075	215731	19.72	42.81	114761	640.1
4-way interactions	41	3.28	1.41	26425	186769	20.1	44.39	124580	445.4
5-way interactions	20	2.16	1.44	11554	25334	11.88	12.14	46918	243.2
6-way interaction	4	0.12	0.08	1088	2560	2.64	0.76	4364	28.8
Residuals	1144	354.64	80.08	1296152	5562128	960.96	3981.12	4554264	68411.2

TM= tillage management; CS= cropping sequence; GCM=ground cover management; AC= Alley cropping configuration; ACe= Local effect of the alley cropping configuration; Sde= soil depth; ΔSOC= Soil organic carbon; ΔPOMC= Particulate organic carbon; ΔPOXC= Permanganate-oxidizable carbon; ΔMBC= Microbial biomass carbon; ΔOls P= Olsen phosphorus; ΔMBP= Microbial biomass phosphorus; ΔNtot= Total nitrogen; ΔMBN= Microbial biomass nitrogen

Table 6: Effects of the main factors on the variation of selected soil properties over the three-year period. Results are expressed as the means with standard errors (in parentheses) Different letters denote statistically significant differences among the levels of each factor.

Variability source	Levels	ΔSOC ($g\ kg^{-1}$)	ΔPOM-C ($g\ kg^{-1}$)	ΔPOX-C ($mg\ kg^{-1}$)	ΔMBC ($mg\ kg^{-1}$)	ΔOls P ($mg\ kg^{-1}$)	ΔMBP ($mg\ kg^{-1}$)	ΔNtot ($mg\ kg^{-1}$)	ΔMBN ($mg\ kg^{-1}$)
TM	Plough	0.26 (0.02) a	0.03 (0.01) a	-5.15 (1.36)	28.3 (2.97) b	0.09 (0.03) a	0.56 (0.08) b	1.76 (2.63)	1.8 (0.33) b
	Red	0.17 (0.02) b	0.00 (0.01) b	-7.04 (1.31)	44.43 (3.29) a	-0.02 (0.04) b	1.06 (0.09) a	-4.72 (2.5)	3.35 (0.38) a
CS	CONTIN	0.23 (0.03)	0.01 (0.01)	-6.6 (1.34)	31.85 (3.22) b	0.02 (0.04)	0.63 (0.09) b	1.74 (2.54)	1.89 (0.37) b
	ROT	0.20 (0.02)	0.02 (0.01)	-5.59 (1.33)	40.88 (3.06) a	0.05 (0.04)	0.99 (0.09) a	-4.71 (2.59)	3.25 (0.34) a
GCM	CC	0.27 (0.02) a	0.04 (0.01) a	-6.47 (1.3)	49.97 (3.5) a	0.09 (0.04) a	1.17 (0.10) a	-2.41 (2.6)	3.82 (0.39) a
	nCC	0.16 (0.02) b	-0.01 (0.01) b	-5.72 (1.36)	22.76 (2.65) b	-0.02 (0.04) b	0.45 (0.07) b	-0.56 (2.53)	1.33 (0.31) b
AC	Mono	0.21 (0.02)	0.02 (0.01)	-5.33 (1.58)	35.91 (4.03)	0.01 (0.04)	0.81 (0.11)	-4.44 (3.26)	2.59 (0.45)
	Hazelnut	0.21 (0.03)	0.02 (0.01)	-7.40 (1.64)	38.71 (3.52)	0.07 (0.05)	0.76 (0.10)	2.08 (2.95)	2.69 (0.41)
	Olive	0.22 (0.03)	0.00 (0.01)	-5.56 (1.68)	34.47 (4.00)	0.02 (0.05)	0.87 (0.11)	-2.08 (3.21)	2.44 (0.44)
ACe	Border	0.22 (0.02)	0.02 (0.01)	-5.36 (1.53)	37.33 (3.19)	0.02 (0.04)	0.83 (0.09)	0.02 (3.03)	2.62 (0.36)
	Center	0.20 (0.03)	0.01 (0.01)	-6.83 (1.11)	35.40 (3.11)	0.05 (0.03)	0.79 (0.11)	-2.98 (2.00)	2.53 (0.35)
Sde	0-5 cm	0.22 (0.03)	0.04 (0.01)	-8.09 (1.35) b	50.88 (3.77) a	0.18 (0.06) a	1.10 (0.11) a	0.05 (3.18)	3.68 (0.43) a
	5-10 cm	0.22 (0.03)	0.01 (0.01)	-13.08 (1.72) b	43.94 (3.69) a	-0.14 (0.04) c	1.12 (0.10) a	-3.36 (3.14)	3.11 (0.44) a
	10-20 cm	0.19 (0.02)	0.00 (0.01)	2.89 (1.71) a	14.28 (3.88) b	0.07 (0.04) b	0.21 (0.10) b	-1.13 (3.12)	0.93 (0.42) b

TM= tillage management (conventional [Plough] vs reduced tillage [Red]); CS= cropping sequence (Continuous cereal cropping [CONTIN] vs Cereal-legume rotation [ROT]); GCM=ground cover management (cover crops [CC] vs. no cover crop [nCC]); AC= Alley cropping configuration (hazelnut-based [Haz] and olive-based [Oliv] alley cropping systems and herbaceous monoculture [Mono]); ACe= Local effect of the alley cropping configuration (border side of the plot [Border] vs center of the plot [Center]); Sde= soil depth; ΔSOC= Soil organic carbon; ΔPOMC= Particulate organic carbon; ΔPOXC= Permanganate-oxidizable carbon ΔMBC= Microbial biomass carbon; ΔOls P= Olsen phosphorus; ΔMBP= Microbial biomass phosphorus; ΔNtot= Total nitrogen; ΔMBN= Microbial biomass nitrogen

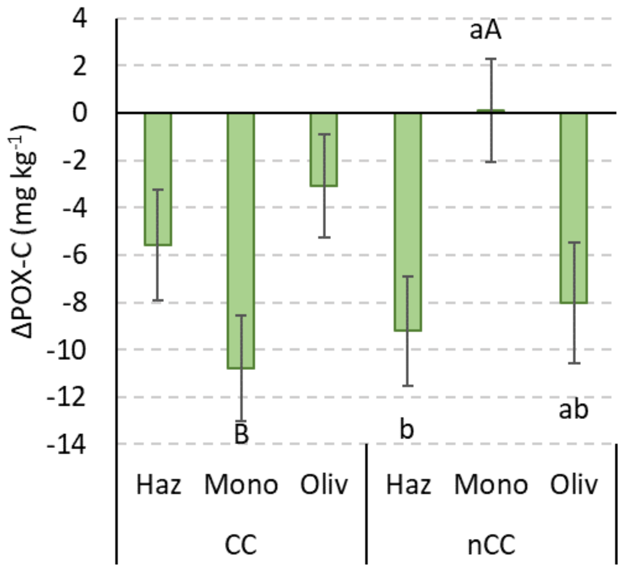

Figure 11: Variation of permanganate-oxidizable carbon (ΔPOX-C) across alley cropping (AC) configurations (hazelnut-based [Haz] and olive-based [Oliv] systems and herbaceous monoculture [Mono]) and ground cover treatments (cover crop [CC] vs no cover crop [nCC]). The results are expressed as the means with the bars representing the standard error. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). Lowercase letters comparing CC within each system and uppercase letters comparing AC within each GCM.

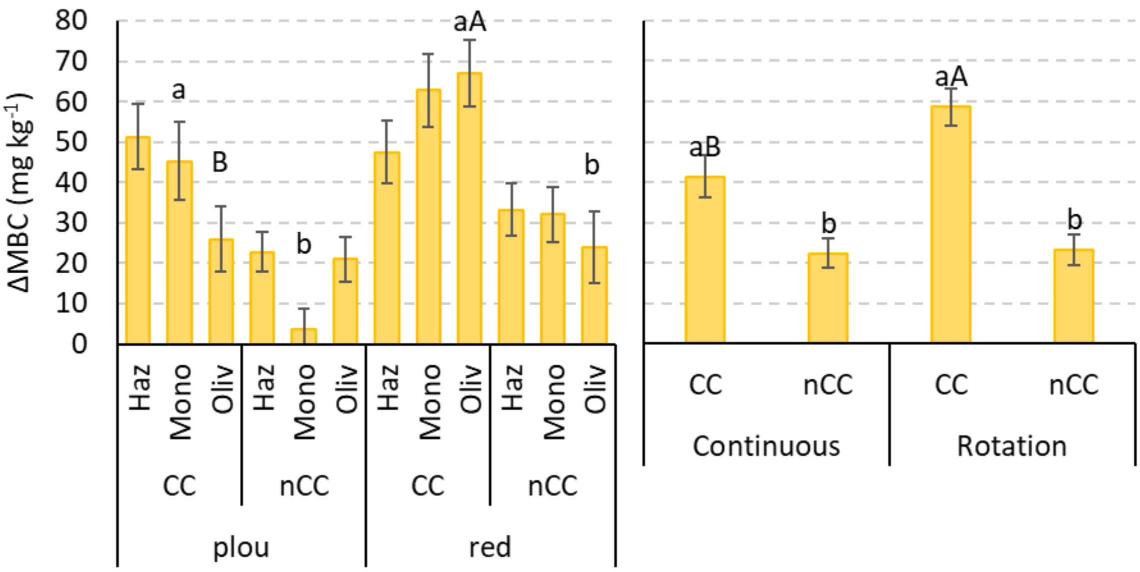

Figure 12: Effect of the interaction between cropping sequence and ground cover management (CS × GCM) on the differential of microbial biomass carbon (ΔMBC) over the three-year period. The results are expressed as the means with the bars representing the standard error. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). Lowercase letters indicate differences between GCM

(cover crops [CC] vs no cover crops [nCC]), while uppercase letters report differences between CS (Continuous cereal cropping [CONTIN] against Cereal-legume rotation [ROT]).

Changes in plant-available phosphorus (measured as Olsen P) were significantly affected by both TM and GCM, while spatial factors such as AC and ACe showed no significant effect (Table S2). Specifically, plough tillage resulted in a net increase of Olsen P, whereas reduced tillage led to a slight decline. Similarly, CC increased Olsen P, in contrast to a minor decrease under nCC conditions. Soil depth had a strong influence ($p < 0.001$). The largest positive change occurred in the 0–5 cm layer, followed by the 10–20 cm, while the 5–10 cm layer experienced a decrease (Table 6).

ΔMBP was significantly influenced by TM, CS, and GCM, with plough tillage resulting in significantly lower ΔMBP values compared to reduced tillage ($p < 0.001$) (Table S2 and Table 6). Similarly, ΔMBP was significantly higher under ROT and CC compared to CONTIN and nCC, respectively ($p < 0.001$ for both) (Table 6) Significant two-way interactions were observed between TM and AC ($p < 0.01$) and between TM and CS ($p < 0.01$). Within the Oliv alley cropping system, ΔMBP under reduced tillage was 2.5 times greater than under Plough (Figure 13A). Under Mono, the increase was 92.6%. In terms of CS, ΔMBP under Plough was approximately four times greater in the ROT than in the CONTIN (Figure 13B). Moreover, within the CONTIN, Red resulted in a ΔMBP nearly five times greater than that under Plough.

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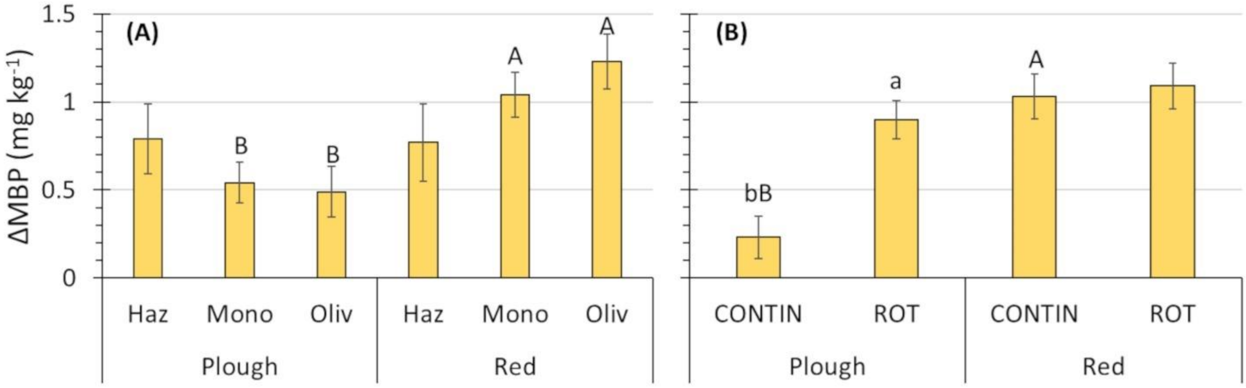

Figure 13: Effect of the interaction between **(A)** tillage management and alley cropping configuration (TM × AC) and **(B)** tillage management and cropping sequence (TM × CS) on the differential of microbial biomass phosphorus (ΔMBP) over the three-year period. The results are expressed as the means with the bars representing the standard error. Different letters denote statistically significant differences among treatments based on the Tukey's HSD post-hoc test ($p < 0.05$). In TM × AC, uppercase letters report differences between TM (reduced [Red] vs conventional [Plough] tillage). In TM × CS, lowercase letters indicate differences among CS (Continuous cereal cropping [CONTIN] against Cereal-legume rotation [ROT]), while uppercase letters between TM.

The ΔNtot did not vary significantly with any of the studied agronomic practices or their interactions (Table S2). Mixed-effects analysis yielded no significant differences ($p > 0.05$) across TM, CS, GCM AC and soil depth. Averaged across all treatments, ΔNtot values oscillated around neutrality, with TM and CONTIN showing slight positive changes, while Red and ROT treatments showed slight decreases (Table 6). Neither CC presence nor AC significantly influenced nitrogen dynamics. As expected, a minor variation with depth was observed, but no consistent trend emerged. These results indicated that ΔNtot remained largely stable over the duration of the study, regardless of the combination of diversified and intensive practices implemented.

The ΔMBN responded significantly to TM, CS, and GCM (Table S2). Red exhibited a notably higher ΔMBN compared to Plough, while ROT boosted ΔMBN relative to CONTIN cropping (Table 6). The

CC plots also showed higher ΔMBN compared to nCC plots. Interactions revealed that under Plough, ROT produced over a four-fold increase in ΔMBN compared to CONTIN, and under CONTIN, reduced tillage resulted in approximately a five-fold greater ΔMBN than Plough (Figure 14).

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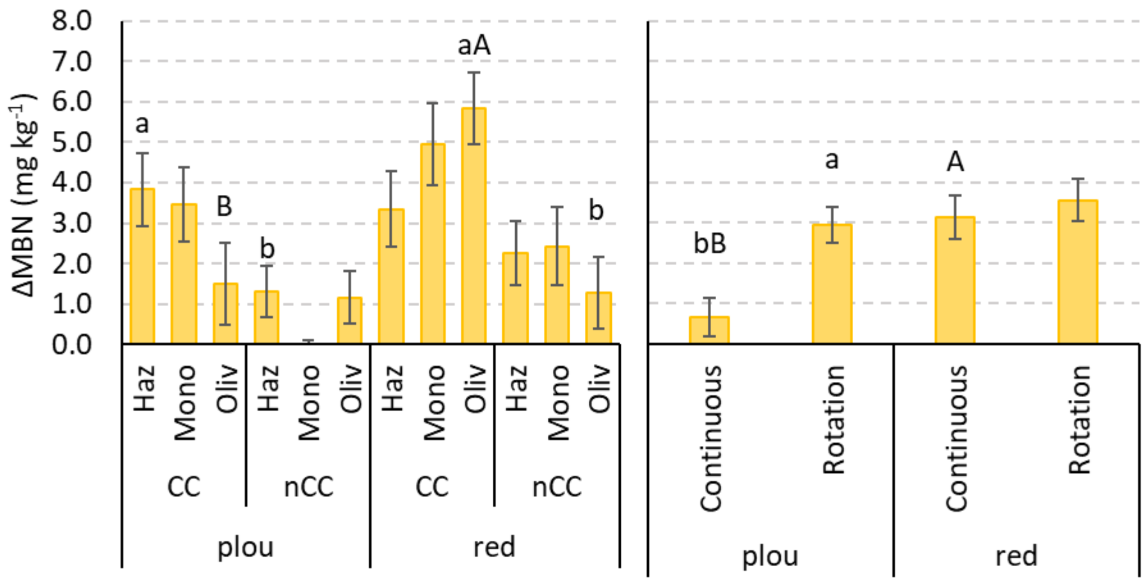

Figure 14: Effect of the interaction between tillage management and cropping sequence (TM × CS) on the differential of microbial biomass nitrogen (ΔMBN) over the three-year period. The results are expressed as the means with the bars representing the standard error. Different letters denote statistically significant differences among treatments based on Tukey's HSD post-hoc test ($p < 0.05$). Lowercase letters indicate differences between CS (Continuous cereal cropping [CONTIN] against Cereal-legume rotation [ROT]), while uppercase letters report differences between TM (reduced [Red] vs conventional [Plough] tillage).

7.5. Discussion

The research from the present study was shown to advance our understanding of how the short-term integration of multiple sustainable agricultural practices, such as reduced tillage, CS, GCM, and AC using agroforestry systems, affects both crop performance and key soil properties associated with soil health. The study offers a multidimensional perspective, encompassing not only yield and nitrogen use efficiency but also shifts in soil organic carbon, microbial biomass, and nutrient dynamics, thus providing relevant insights for more resilient and resource-efficient farming systems.

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7.5.1. Pre-crop effect- Spring Barley performances

Sowing TR as a CC represents a simple and easily adoptable practice for farmers, requiring minimal modifications to their existing cropping systems, particularly in systems with extended fallow periods between main crops. Use of TR as a winter cover crop led to a significant increase in spring barley GY, consistent with findings in winter wheat systems where radish and other cover crops boosted yields by 27–32% (Garbellini et al. 2022). This yield boost is commonly linked to disease suppression and improved soil health (Garbellini et al. 2022) as well as enhanced phosphorus availability (Pescatore et al. 2025a; Pescatore et al. 2025b). The significant interaction between tillage and growing season underscores the variable response of GY to soil disturbance under different climatic conditions. During GS2, plough tillage yielded higher GY than reduced tillage, suggesting conventional tillage may enhance early root development and nutrient access. Yield reductions linked to the initial shift to conservation agriculture are documented in the literature (Kihara et al 2012; Van den Putte et al. 2010). Although in GS3 ploughing maintained a 34.9% yield advantage over reduced tillage, the gap narrowed, likely due to early-season waterlogging associated with above-average March rainfall. Waterlogging compromises ploughed soil, attributable to poor drainage and reduced aeration, whereas reduced tillage systems appear more resilient. Comparable resilience of reduced tillage under excess moisture has also been observed in other crops (Vlček et al. 2022).

SY responded significantly to the interaction between tillage and ground cover, with the highest SY observed under the combination of plough tillage and CC. Seasonal variability in SY mirrored trends for GY, with GS2 outperforming GS3, although CC notably mitigated GS3 losses, potentially via biopore formation improving soil aeration and drainage (Zhang et al. 2021). PY was similarly enhanced by the CC, indicating improved nitrogen availability for barley, consistent with previous radish-cover studies (Elhakeem et al. 2023). While plough tillage generally resulted in higher PY, the disparity between plough and reduced tillage diminished in GS3, again reflecting reduced tillage's adaptive benefits under waterlogged conditions. The adverse impact on GY by waterlogging during vegetative growth stages is well-documented (Ren et al., 2017).

7.5.2. Cropping systems performances

The analysis of cropping system performances integrated multiple sustainable practices, TM, CS, GCM, and AC using agroforestry systems, to evaluate their combined potential for enhancing resource use efficiency and crop productivity. These aspects can guide farmers in selecting specific combinations of agricultural practices that not only maximize productivity but also support the provision of ecosystem services. During the initial years of transition to reduced tillage, the EY under plough tillage remained superior to that under reduced tillage (Van den Putte et al. 2010). However, the incorporation of ROT, particularly with red clover, mitigated this yield gap. Red clover has been shown to buffer the negative impacts of reduced tillage on crop performance (Chehade et al. 2023) and enhance the performance of subsequent cereal crops (Kirkegaard et al. 2008). The substitution of spring barley with red clover during GS2 likely contributed to the observed EY improvement. Moreover, herbaceous crop Mono and Oliv-based AC exhibited the highest EY during transition, whereas Haz-based AC yielded the lowest, confirming competitive shading effects from hazelnut trees (Braun et al. 2011).

NUE in terms of NUE_{synt-e} defined as the ratio of harvested energy to synthetic N input, showed clear patterns across management strategies. Overall, the highest NUE_{synt-e} was highest under plough tillage and ROT, while recently transitioned reduced-tillage systems exhibited lower values, consistent with documented transient yield declines during such transitions (Arvidsson et al. 2014). When ROT was applied, NUE_{synt-e} under herbaceous Mono surpassed that in the Haz-based AC, reaffirming the stronger competitive impact of hazelnut trees on intercrops. In contrast, the Oliv-based AC attained NUE_{synt-e} values comparable to that of Mono, indicating more synergistic interactions with annual crops. Notably, in the absence of ROT, differences in NUE_{synt-e} across cropping systems disappeared, emphasizing that combining ROT with AC agroforestry was critical to optimize N use, as also reported by Xia et al. (2024). Moreover, TBM peaked under plough tillage. However, the use of TR as a CC significantly enhanced TBM relative to nCC, corroborating findings by Debiasi et al. (2025) in a soybean–wheat cropping system.

$NUE_{synt-p,}$ calculated as the ratio of harvested protein to synthetic N applied, varied substantially among cropping systems. Within ROT, both the herbaceous Mono and Oliv-based AC achieved the highest PYs, whereas the Haz-based AC lagged significantly. These differences were minimal under CONTIN, likely due to the uniformly high N demand of the successive cereal crops. The inclusion of legumes in ROT appeared instrumental in enhancing nitrogen availability and supporting protein accumulation, as reported by Simon-Miquel et al. (2025) in diversified continuous maize systems.

These findings underscore the importance of legume integration, even within already diversified AC systems, to enhance N availability and overall system performance.

The NUE_{synt-p} was strongly influenced by the interaction between cropping system and crop rotation. The highest NUE_{synt-p} values were observed in systems where ROT included legumes. Systems with legumes exhibited the highest efficiencies, reflecting their enhanced capacity to convert synthetic N into protein. It should be noted that NUE_{synt-p} excluded biologically fixed N, yet this remains a valuable metric of how effectively a system minimizes synthetic inputs while maximizing protein output. In hazelnut alleys, typically constrained by tree–herbaceous competition (Brambila et al. 2025), the inclusion of legumes significantly improved NUE_{synt-p}, indicating that legumes can partly offset competition. However, in the present study, the Haz-based AC continued to underperform compared to Mono and Oliv-based AC, corroborating the superior compatibility of olive with Mediterranean agroecosystems (Aguilera-Huertas et al. 2024). The present results were also consistent with the documented high nitrogen demand of young hazelnut orchards (Pacchiarelli et al. 2022).

7.5.3. Soil response to the adoption of different sustainable practices

Although changes in soil properties typically require long-term monitoring, short-term responses can still provide valuable early indicators of soil health dynamics. Over the three-year transition from a conventional cereal monoculture system to diversified management, including CC, CS, TM, and AC, we observed that ΔSOC concentration responded significantly to plough tillage and the use of TR as a CC. Contrary to studies that reported increased SOC under reduced tillage (Swanepoel et al. 2018; Jayaraman et al. 2022), our results indicated higher SOC levels with conventional tillage. This aspect was likely linked to a greater biomass production, a known driver of SOC accumulation

(Prommer et al. 2019). Additionally, plots with TR as a CC exhibited significantly greater SOC accumulation than nCC. This finding corroborated recent studies demonstrating that radish-based systems substantially boost SOC in the topsoil (Wang et al. 2024; Pescatore et al. 2025a; Pescatore et al. 2025b). To the best of our knowledge, this is the first demonstration of TR-induced SOC improvement in a cereal-based cropping system. The robustness of this result is supported by a large sample size and suggests early signs of improved soil quality following the adoption of TR as a CC.

POM-C, a labile fraction of SOC (primarily derived from crop residues and partially decomposed plant materials) mirrored the overall trend in SOC, underscoring its sensitivity to management practices. This result was anticipated as POM-C predominantly originates from crop residues, which serve as primary inputs to this pool (Si et al. 2024; Derrien et al. 2023). However, the POX-C, another labile SOC fraction, was not significantly affected by treatment variables such as TM and CS. The only significant impact was soil depth, with POX-C increasing at greater depths regardless of management strategy. This pattern likely reflected a reduced microbial activity in subsoil horizons, which slows decomposition of this biologically active carbon pool (Culman et al. 2012).

The MBC declined significantly with soil depth and was markedly higher in plots sown with TR, corroborating findings by Torabian et al. (2025), who reported increased MBC following radish cover crops in a potato system. Interestingly, the increase in MBC was even more pronounced when TR was combined with legume rotations, highlighting their synergistic role in stimulating microbial activity and contributing to improved soil health.

Similarly, plots with TR exhibited elevated Olsen P compared to nCC treatments, echoing improvements in P availability reported by (White and Weil 2011), even in low-P soils. MBP was significantly influenced by the interaction between TM and cropping systems, likely due to greater soil moisture retention as noted by Xiao et al. (2024). However, in Haz-based AC systems, no significant TM effect was observed, possibly because the high-water demand of hazelnut trees has been shown to offset moisture differences (Vinci et al. 2023), which in turn may have offset the moisture conservation benefits of reduced tillage. The lowest ΔMBP occurred under plough tillage with CONTIN, indicating poor microbial P immobilization in such systems. These results were consistent with previous findings, demonstrating enhanced microbial P in cereal–legume systems under Mediterranean conditions (Tang et al. 2014).

Present resulted showed that the values of ∆MBN mirrored that of ΔMBP. Consistent a previous meta-analysis (Xing et al. 2022), which found crop rotation enhances MBN, our results showed significantly higher MBN under reduced tillage and in rotated systems. Under plough tillage, ROT increased MBN more than fourfold compared to CONTIN. Moreover, under CONTIN, reduced tillage resulted in approximately fivefold higher MBN than under plough tillage.

7.6. Conclusion

The present study evaluated the short-term effects of integrating multiple sustainable agricultural practices into a conventional cereal monoculture under Mediterranean rainfed conditions. The introduction of TR as a winter CC between main crop cycles was associated with notable improvements in GY and PY of the subsequent spring barley. At the cropping system level, the combined adoption of ROT and Oliv-based AC were shown to enhance TBM, PY, and NUE (both in terms of energy and protein), attaining levels comparable to those of the Mono. Concurrently, this system promoted key indicators of soil health, including increases in SOC, POM-C, and MBC. TR sowing also significantly increased Olsen P and MBP, highlighting the capacity of this CC to improve P availability and potentially reduce reliance on synthetic P fertilizers.

Overall, these findings underscore the synergistic potential of combining multiple sustainable practices to simultaneously enhance crop productivity and soil quality. Further research is warranted to assess the long-term impacts of these integrated strategies across a wider range of environments, soil types, and cropping systems to guide their effective implementation and broader adoption.

7.7. References

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8. General conclusions

This Ph.D. thesis explored the potential of multiple cropping systems, particularly those integrating cover crops, to enhance soil fertility and sustain cropping system productivity. Two field experiments were carried out in contrasting agroecosystems: a conventional cereal-based system and a lawn-managed system. The study examined how the inclusion of cover crops and other soil-improving practices affects key chemical, biological, and physical soil properties, providing valuable insights into thier implications for crop performance and environmental sustainability.

A screening trial was conducted to assess the effect of different cover crops on key soil health properties. This trial was carried out in a lawn-managed field system, where various clover species and crop mixtures were compared against a conventional monoculture of clover. The study focused on double cropping with cover crops, aiming to evaluate their influence on soil health and carbon sequestration. In particular, this study assessed the impact of various herbaceous crops on aboveground biomass production, soil organic carbon (SOC) dynamics, carbon sequestration, and water retention capacity. The results clearly demonstrated that the choice of herbaceous cover crop species significantly influences all these soil health properties.

Among the tested species, crimson clover produced the highest aboveground biomass, confirming its adaptability and productivity under lawn-based management. However, tillage radish, despite producing less biomass, emerged as the most effective treatment in enhancing key soil health properties. It significantly increased both labile and stable SOC fractions, achieving the highest annual carbon sequestration rate (4.58 t CO_2 ha^{-1} y^{-1}), and enhanced soil water storage capacity. These benefits are likely attributable to the tap-root system of tillage radish (TR), which enhanced soil porosity and introduces root-derived carbon. In contrast, grass-based or mixed herbaceous treatments showed significant improvements and, in some cases, even resulted in net carbon loss. This suggests that a single growing season may be insufficient for such mixtures to significantly enhance soil functions, especially in conservation agriculture. Overall, the findings highlight the importance of species selection in optimizing lawn-managed field for climate mitigation and water resilience objectives. While crimson clover proved effective for biomass production, tillage radish demonstrated multifunctionality and strong potential for sustainable land management.

Given the exceptional performance of tillage radish in carbon sequestration and soil water storage, further research was conducted considering the integration of tillage radish into other agroecosystems. Its ability to rapidly improve soil conditions makes it a promising candidate for conservation and regenerative agricultural practices and nature-based solutions in different agroecosystems. Therefore, tillage radish was further evaluated in a conventional cereal-based cropping system. This second study provides comprehensive insights into the performance of tillage radish under contrasting tillage systems and growing seasons in the Mediterranean context. In general, tillage radish produced more biomass, mobilized more nutrients, and improved soil quality indicators more effectively under conventional tillage than under reduced tillage. These effects were attributed to improved root development and nutrient cycling facilitated by greater soil disturbance.

Conversely, under reduced tillage, tillage radish growth and nutrient uptake were consistently limited, particularly during drier conditions, likely due to restricted root penetration and delayed organic matter mineralization. Seasonal differences also played a significant role, with wetter seasons promoting shoot growth and nutrient translocation, while drier periods favored greater root allocation.

Tillage radish also improved soil organic carbon (SOC), labile carbon fractions, microbial biomass, and nutrient availability, particularly under conventional tillage. These improvements were more pronounced in deeper soil layers, indicating that tillage radish residues can influence subsoil carbon and nutrient dynamics. However, under reduced tillage, the limited biomass input and slower decomposition hindered these improvements, underscoring the need for tailored management strategies.

Overall, tillage radish demonstrated its potential as a multifunctional cover crop for improving soil fertility, enhancing carbon and nutrient cycling, and supporting sustainable soil management in degraded systems. Nevertheless, its suboptimal performance under reduced tillage revealed important knowledge gaps. Evaluating its use across different conservation systems proved essential to inform management adaptation.

Building on these findings, a broader investigation was conducted to assess the agronomic and ecological performance of tillage radish under different pedoclimatic and management conditions.

The results underscore the importance of site-specific factors, such as soil texture, compaction, and climate, in determining the effectiveness of tillage radish. For instance, higher biomass and nutrient uptake were observed in locations with finer soil texture, lower salinity, and more favorable rainfall and temperature conditions.

The study also revealed the ability of tillage radish in promoting labile soil organic carbon fractions (ΔPOM-C, ΔPOX-C) and microbial biomass pools (ΔMBC, ΔMBP, ΔMBN), with greater enhancements associated with higher biomass accumulation. These results align with existing literature on the importance of biomass thresholds and residue quality in driving soil health improvements. However, limited biomass in cereal-based system constrained benefits, particularly in heavier soils, reinforcing the need for improved residue and soil structure management.

While tillage radish demonstrated a strong potential for nitrogen retention and microbial nitrogen accumulation, the study also identified risks of nitrogen leaching, expecially under heavy rainfall or poor residue management. This dual role underscores the importance of carefully balancing benefits and risks through site-specific strategies.

Overall, the results confirm the role of tillage radish as a versatile tool in conservation agriculture, particularly in enhancing soil health indicators related to carbon, nitrogen, and phosphorus cycling. While its positive impacts on soil biology and chemistry were clear, its effect on subsequent crops remains a knowledge gap.

To address this, a complementary study assessed how the short-term integration of sustainable agricultural practices, including tillage radish cover cropping, affects both soil and crop performance in Mediterranean cereal systems. The findings demonstrated that practices such as reduced tillage, cover cropping, crop rotation, and alley cropping, when combined with tillage radish, can enhance soil quality and crop performance. The sowing of tillage radish between crop cycles enhanced the grain and protein yield of spring barley, likely due to improved nutrient availability and soil structure. Plough tillage initially yielded higher outputs, but reduced tillage showed more resilience under waterlogged conditions, suggesting long-term structural benefits.

At the system level, rotation and olive-based alley cropping provided yields comparable to monoculture, while improving nitrogen use efficiency and soil properties. Conversely, hazelnut-based alley cropping reduced herbaceous crop performance due to competition, although legume integration lhelped mitigate these effects. From a soil perspective, tillage radish improved SOC, POM-C, and microbial biomass, indicating rapid gains in soil quality. It also increased phosphorus availability (Olsen P, microbial P), even in low-P soils.

These findings show that even short-term adoption of multiple sustainable practices can enhance productivity, resource use efficiency, and soil health. Future research should explore long-term effects and scalability across Mediterranean cropping systems.

In light of these findings, the relevance of this research extends beyond the scientific domain. It aligns with the objective of the Common Agricultural Policy (CAP 2023–2027), which supports practices such as soil cover, reduced tillage, and crop diversification. By generating robust, field-based evidence, this research supports evidence-based policymaking and highlights multiple cropping systems as effective strategies for soil conservation, biodiversity enhancement, and climate mitigation.

Throughout this research, stakeholder engagement and dissemination activities were prioritized. The cereal-based experiment was embedded within a Living Lab framework, fostering collaboration between researchers, farmers, and advisors. Results were actively shared through field days, stakeholder workshops, and technical meetings, promoting knowledge exchange and encouraging the practical adoption of sustainable practices. These efforts not only increased the visibility and applicability of the research but also contributed to bridging the gap between scientific innovation and practical agriculture, supporting the transition toward more resilient and climate-smart farming systems.

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