



Influence of Crop Rotation Complexity on Soil Health in Organic Greenhouse Production

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Influence of Crop Rotation Complexity on Soil Health in Organic Greenhouse Production

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Keywords: *Cucumis sativus*, intercropping, legumes, organic greenhouse production, *Pisum sativum* var. *saccharatum*, soil health

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Abstract

This study evaluated the hypothesis of greater crop rotation complexity leads to improve the soil biological health. According to European Union (EU) requirements for organic production (EU Regulation 2018/848), organic production must be take place directly in the soil, and a crop rotation is mandatory. Additionally, with the crop rotation must include a green manure crop that can add nutrients to the soil. Two crop rotation systems were compared; one had a simple crop rotation using nitrogen fixing crop (sugar pea; *Pisum sativum* var. *saccharatum*) and the other had crimson clover (*Trifolium incarnatum*) intercropping with cucumber. Soil samples were taken from each system over time to measure soil respiration, microbial activity, total protein, active carbon, organic matter and available nutrients in the soil. The overall time effect proved highly significant ($P < 0.01$) for soil active carbon, total protein content, total organic matter content, phosphorus content, and microbial activity; therefore, time appeared to be the main driving factor behind the dynamics of studied biological and chemical properties of soil. As for the influence of crop rotation, it turned out to be relatively minor since only potassium content exhibited a significant rotation effect ($P < 0.05$). Moreover, interactions between crop rotation and time also appeared to have a significant influence ($P < 0.01$) on pH value, electrical conductivity, nitrogen content, and soil respiration rate ($P < 0.05$), thus making the influence of crop rotation dependent on time and vice versa. Therefore, crop rotation appeared to be a comparatively less effective aspect affecting soil health.

Keywords: Crop complexity, *Cucumis sativus*, European Union, Legumes, Microbial activity, Nitrogen fixing, *Pisum sativum* var. *saccharatum*, Temporal variation, *Trifolium incarnatum*.

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Abbreviations

Abbreviation	Description
BCA	Bicinchoninic Acid
BSL	Bio Safety Level
EU	European Union
SLU	Swedish University of Agricultural Sciences
SOM	Soil Organic Matter

1. Introduction

Soil is one of the key natural resources that plays an important role in supporting plant growth, nutrient cycles, and water regulation. Soil health is one of the most critical factors in sustainable farming practices. Sustainable environment and ecosystem operation largely depend on the health of the soil. Healthy soils always contain plenty of nutrients, organic matter, microbes which helps to decompose organic matter, nutrient cycling and for nitrogen fixation like functions while increasing crop productivity and reducing environmental degradation (Doran & Zeiss 2000).

In the recent years, greater attention has been given to soil biological indicators due to their quick reaction to the changes in the environment and management practices than physical and chemical soil attributes. Soil microorganisms involved in the process of decomposition of organic matter and nutrient cycling making them important components of soil health assessment (Kracmarova et al. 2022).

The crop rotation is viewed as the most important agricultural practice. The introduction of various crops through rotations can lead to increased microbial biomass in the soil as well as enhanced soil biological functioning than under the traditional monoculture farming practice in which the same crop is cultivated on the in same land continuously throughout all growing seasons without diversification (Kracmarova et al. 2022). World population will continue increasing, and the need for food production from agriculture will increase further. According to the Food and Agriculture Organization (FAO), food production needs to be increased considerably before 2050 to meet demands (FAO, 2009). Therefore increasing crop productivity while sustaining the environment is crucial factor.

Temporal changes of soil health parameters like soil microbial activity, soil active carbon, soil protein, and soil respiration are critical in understanding dynamics of soil health with regard to agricultural practices. Monitoring variations in soil health parameters over time is crucial in gaining knowledge about the sustainability of different crop rotations and its effects on soil improvement. With all these perspectives, the current study was done to evaluate how temporal variation and two different crop rotation systems with legumes effect on specific soil health parameters.

2. Research Background

2.1 Soil Health

2.1.1 Definition

Soil is generally viewed as the uppermost portion of the Earth's crust that facilitates plant vegetative growth, by allowing roots to access nutrients (Brady & Weil 1996). “The soil health can be described as Soil health is the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health (Doran & Zeiss 2000).

Simultaneously, according to the United States Department of Agriculture (USDA) definitions, it can be characterized as “capacity of soil to provide essential ecosystem services such as cycling of nutrients, water retention and regulation, and biodiversity conservation. Soil health directly reflects the overall condition of the soil ecosystem” (USDA NRCS, 2018).

2.1.2 Soil Health vs Soil Quality

Concepts of soil health and soil quality are different concepts, but interrelated. Soil quality can be described as, its properties that can affect its ability to function as needed and can be measured physically and chemically. (Karlen et al. 1997). Further soil health focuses primarily on the biological systems of soil and the organisms present in soil (Doran & Zeiss 2000). Soil quality can be assessed by using indicators such as soil texture, soil pH, and nutrient levels, which are approximately stable over time (Karlen et al. 1997). Also soil health evaluation is dynamic and includes indicators of microbial function and diversity, which are directly bind with agricultural management such as organic matter inputs, tillage intensity, crop rotation (Kibblewhite et al. 2008). This differentiation is really important when maintaining sustainable organic agriculture production. Soil health is a crucial factor in maintaining ecosystem resilience. Because healthy soils have the ability of recovering, from problems like drought, heavy rain, or soil borne disease, and support healthy plant growth. As an example, organic matter rich soils can maintain good water level in dry period and helps better crop survival even in dry periods. Soil organic matter decomposed material from living organisms like plants, animals, and microorganisms in the soil. It has greater ability to maintain soil health (Lal 2020).

2.1.3 Biological Soil Health as a Key Component

We can define biological soil health as, activities, diversity and function of soil microorganisms, which include bacteria, fungi, and soil fauna (Nannipieri et al. 2003). These microorganisms play a major role in nutrient cycling, decomposition of organic matter, and maintaining soil structure. Soil microorganisms also provide essential components of ecosystem functioning, including nitrogen fixation, mineralization and carbon sequestration. (Van der Heijden et al., 2008).

In organic greenhouse vegetable production, where synthetic fertilizers and pesticides are not allowed to be used, soil health and quality are primarily maintained through biological processes like microbial functions and organic matter content (Lori et al. 2017).

2.2 Soil Health Indicators and Their Importance

Soil Health Indicators are those biological, chemical, and physical attributes of the soil which demonstrate its ability to serve as a living entity. The role of these indicators is to assess the performance of the soil in terms of supporting crop production, biological processes, nutrient recycling, and environmental protection (Karlen et al. 1997; Doran & Zeiss 2000).

2.2.1 Soil Respiration

Soil respiration is commonly used as a strong indicator of microbial metabolic activity, which measure the released carbon dioxide amount (CO_2) from soil as a result of biological processes (Anderson 1982). It is one of the most common indicator for measuring biological activity and overall soil health.

It is important to have balanced soil management to maintain soil respiration. Soil respiration can use as a good indicator, but higher soil respiration cause rapid carbon loss from soil, and rapid organic matter decomposition (Franzluebbers et al. 2000). According to Cornell Soil Health Assessment manual, measuring respiration with trapping emitted carbon dioxide normally gives a rapid cost effective and reliable measurement about microbial activity level (Moebius-Clune, et al., 2017).

2.2.2 Microbial Activity

Soil microbial activity is also a key indicator of soil health (Vance et al. 1987). It also represents the size of active microbial biomass involved in nutrient cycling in soil. A larger microbial diversity is typically associated with improved soil fertility and greater capacity for nutrient transformation. As example, soils with a high and active microbial population helps to rapid decomposition of organic matter and tend to release essential nutrients for plant growth, while soils with

less microbial community tend to have limited nutrient availability to plant growth (Cerri & Jenkinson 1981).

In the Cornell protocol, microbial activity is indirectly assessed through respiration, but additional methods such as chloroform fumigation extraction can provide more detailed insights into microbial biomass (Moebius-Clune, et al.,2017).

2.2.3 Soil Protein

Soil protein, particularly ACE (Autoclaved Citrate Extractable) protein, is an important indicator of soil health. It represents the organically bound nitrogen content in soil (Wright and Upadhyaya, 1998). If higher the soil protein levels, it is higher the nitrogen availability and microbial activity (Hurisso et al. 2018). According to Cornell Soil Health Assessment manual, Autoclaved Citrate Extradable (ACE) protein index represent the large pool of organically bound nitrogen in the soil organic matter, which mineralize and make available for plant uptake. Therefore it is a good indicator for biological and chemical soil health, overall the quality of the soil organic matter. (Moebius-Clune, et al.,2017)

2.2.4 Soil Organic Matter (SOM)

Soil organic matter is also a important component of soil health, influencing soil physical structure, soil nutrient availability, and biological activity (Lal 2020). Soil organic matter serve as a reservoir for nutrients and energy for soil microorganisms. As explained above, higher soil organic matter levels cause improve soil structure, water retention, and cation exchange capacity. In organic systems, maintaining organic matter is essential for sustaining productivity and resilience (Bronick & Lal 2005).

2.2.5 Soil Active Carbon

Active carbon is a good indicator for soil organic carbon. It is readily available portion of carbon that easily available to microorganisms (Weil et al. 2003). It is the primary energy source for the soil microbial community. It often used as a sensitive indicator of changes in soil health management. High active carbon levels means there are good microbial activity level and nutrient cycling (Havlin 2014). According to Cornell Soil Health Assessment manual, measure soil active carbon by using permanganate oxidation, gives a rapid measurement of soil biological activity. Active carbon is positively correlate with available organic matter, aggregate stability and overall microbial biomass.

2.2.6 Soil Available Nutrients

Soil available nutrients, including the macro nutrients nitrogen, phosphorus, and potassium, like macro nutrients and other micro nutrients are essential for plant growth, energy transferring, metabolic activities and productivity (Havlin 2014). These nutrients are readily available by mineralization. The availability of nutrients is commonly measured using chemical extraction methods.

2.3 Importance of These Indicators for Maintaining Soil Health

Biological indicators such as soil respiration, microbial biomass, and active carbon directly provide a comprehensive assessment of soil health. These indicators need to be managed carefully and detected early to identify changes in soil condition. Maintaining microbial activity and diversity ensures efficient nutrient cycling and soil fertility. Similarly it helps to maintain good soil structure, water retention capacity, and finally for the long term sustainability. As an example, in soils that are always disturbed by tillage and by other practices, microbial biomass and active carbon levels tend to decrease (Geisseler & Horwath 2009). As a result of it, slower nutrients release, weakens of soil structure, reduce water holding capacity happens. Meanwhile soils with higher biological activity can maintain better structure by maintain aggregates and pores and supply nutrients more steadily to plants (Herridge et al. 2008). Standardized assessment tools like the Cornell Soil Health Assessment contribute for continuous monitoring and management of soil health.

Maintain soil health in a sustainable way is essential for changing climatic conditions, because climatic changes can alter soil biological, chemical and physical processes, cause to reduce soil fertility. Cover crops, minimizing tillage, and incorporating organic matter helps to improve soil health by increasing soil organic matter content, increasing biodiversity, and improving resilience to bad weather events (Lal 2020). Healthy soils directly influence on agricultural productivity while contributing to achieve environmental goals, such as carbon sequestration and biodiversity conservation (Reganold & Wachter 2016).

2.4 Organic Greenhouse Crop Production

2.4.1 Importance of Organic Greenhouse Production in Europe

Organic greenhouse production is becoming an important sector in the European Union because of increasing year round demand for organically produce vegetables. Although the total cultivated area remains relatively less compared

with inorganic production, the sector has high economical importance because production is mainly focused on high value crops such as tomato, cucumber, and leafy vegetables .

The expansion of organic greenhouse vegetable production, mainly depends on market demand and policy support, especially through commitments and of European Union. According to EU policy framework, organic agriculture plays a vital role on not only for fulfilling consumer demands but also in supporting biodiversity, maintaining healthy soil, and reducing overall environmental impact. (European Union, 2018)

Greenhouse vegetable production plays an important role in Europe because controlled environment agriculture helps ensure continuous production and maintain stable yield while avoiding seasonal variation. Although greenhouse systems are often intensive and require careful management such as input, energy and soil fertility. Therefore organic agriculture become main focus regarding balance productivity while sustain ecology (Tittarelli 2020).

The introduction of Regulation (EU) 2018/848 has marked a significant turning point of implementation of organic greenhouse production in Europe. One of the most significant changes is the soil based production system. The regulation defines that, organic production as being based on living soil in connection with subsoil and bedrock termination of using demarcated beds as a organic greenhouse practice (Tittarelli 2020).

This rule mainly focuses some countries which are previously allowed decarcated beds based systems. Under the new framework, that kind of practices not allowed, with only temporary derogations were allowed in specific Nordic countries like Sweden, Denmark and Finland where demarcated beds had been mainly used for organic greenhouse vegetable production.

The main aim of the regulation is to introduce fundamental principles of organic agriculture, particularly the reliance on ecology and ecological processes rather than simple input substitution. It main objective is to promote natural nutrient cycling, enhance soil biodiversity and maintain long term soil fertility. These changes are cause to enhance mainly the customer trust and system integrity by aligning organic greenhouse production with agroecological principles. (European Union). However, these changes cause significant implications, especially for previous production systems with non-soil based techniques.

Another key requirement is mandatory inclusion of crop rotation and green manure practices as part of maintaining soil health. Under Regulation (EU) 2018/848, soil fertility and biological activity should be maintained by, multi-annual crop rotation, including leguminous crop, use of green manure crop and application of green manure or compost (European Union, 2018).

In greenhouse production systems, traditional crop rotations are considered as constrained due to intensive cultivation practices. Therefore, currently it is

promote that green manure crops and legumes are integrated into cropping systems. This practice can help to increase nitrogen availability, improve soil structure, and promote microbial activity and suppressing pest and diseases (Tittarelli 2020).

From an agro ecological perspectives, green manure crops contribute to nutrient recycling and disease suppression, like eco system services. Implementing challenges are limited space, high crop value and continuous production cycles (Drinkwater et al. 1998).

2.5 Challenges for Nordic Greenhouse Systems

Meanwhile organic greenhouse production in Nordic countries faces unique climatic contains like short growing seasons, less light intensity and cool temperatures. Considering Nordic greenhouse systems, production is highly intensive and focused on continuous cultivation of high value crops, which can limit opportunities for crop rotation and green manure integration compared to some Southern European systems like Spain and Italy (Alsanius et al. 2019). When moving into soil based systems, soil borne diseases become major constraint in organic green house production. Limited options of crop rotation, continuous cropping can cause rapid buildup of pathogens such as root rot, nematodes and wilting diseases. Organic systems are really vulnerable because in here chemical control options are restricted, crop rotations often should be short and simplified and input of high nutrients can favor proliferation of pathogens (Alsanius et al. 2019).

Studies show that soil borne pathogen can accumulate quickly in greenhouse soil when compared with open fields because of continuous limited crop rotation and warm environmental condition. This leading significant yield losses if not properly managed. Therefore in organic systems, maintaining soil health relies mostly on biological processes than the chemical intervention .

To overcome these issues, there is critical need for innovative soil management strategies to organic greenhouse systems, specially for all soil based systems. Improving soil fertility and biological activity needs to shift from input based approaches to system redesign basically on agro ecological principles. These include use of agro ecological service crops, improved compost and organic amendment management, biological control of soil pathogens, and enhance crop rotation design. However implementing all these strategies in greenhouse systems is tricky due to high cost, economic constraints, and due to environmental limitations. Tittarelli (2020) highlight that maintaining soil health while ensuring profitability is the one of main challenges of European organic greenhouse vegetable production .

2.6 Crop Rotation, Soil Health, and Inter-cropping Systems

Crop rotation is basically a planned sequence of growing different crops on the same land with time instead of cultivating the same crop whole over the year. This is widely used practice in sustainable and organic agriculture specially to maintain soil fertility, break pest and disease cycles, and improve overall soil health (Bullock 1992; Munkholm et al. 2013). Diverse crop rotations can improve of soil structure and aggregation helps better root penetration and water infiltration through soil (Bronick & Lal 2005). Moreover crop rotation also increases organic carbon stock of soil which cause mitigating climatic changes and maintain soil fertility (Lal 2020).

Crop rotation also influences not only on physical soil properties but also for increasing biological properties, particular soil microbial dynamics (Philippot et al. 2013). These interactions improve microbial diversity and it promote interactions between beneficial microorganisms (Gamliel & Van Bruggen 2016).

Legumes are one of the main component of crop rotation systems due to their ability of fixing nitrogen available in atmosphere through symbiosis with rhizobia bacteria (Peoples et al. 2009). Fixing of biological nitrogen will significantly reduces the need of additional fertilization in organic production (Herridge et al. 2008). The nitrogen availability from legumes becomes available to main crops through residue decomposition of the legumes (Drinkwater et al. 1998). Legumes contribute to improve soil microbial activity by releasing organic compounds which cause to stimulate microbial growth. According to Philippot et al., 2013 findings, plants release organic compounds like carbohydrates, organic acids, and amino acids that can be work as energy sources for microorganisms live in soil (rhizosphere). Moreover legumes works for stimulate nitrogen fixation of specific beneficial microbes like symbiotic Rhizobia for and nutrient uptake from mycorrhizal fungi by using specialized signaling compound like flavonoids (Philippot et al. 2013). These are very important processes considering organic farming due limited fertilizer application.

Green manure crops widely use in organic systems specially to maintain soil fertility and for maintaining overall soil health. Incorporating green manure crops into the soils, it helps to provide organic matter and help recycle nutrients by retaining residual nutrients that can be lost through leaching (Thorup-Kristensen 2006). In addition green manure crops also serve as weed suppressor control of erosion and overall sustainability of the production (Snapp et al. 2005).

Crop rotation complexity plays a crucial role in determining their effectiveness and efficiency (Gaudin et al. 2015). Simple rotations with only two crops normally provide less number of benefits compared to complex systems with multiple crop species by enhance soil fertility, improve structure, and reduce pest

and disease pressure through increased functional diversity (Smith et al. 2008). Increasing crop rotation diversity, eco system multi-functionality, nutrient cycling and pest controlling also automatically enhanced (Letourneau et al. 2011). According to Li et al., 2014, by growing more crops simultaneously rather cultivating one crop at once, it improves resource use efficiency because different crops utilize light, water, and nutrients in different ways, reducing competition and enhancing overall system productivity. As an example, where legumes release organic acids which cause to increase availability of phosphorus for subsequent crops. In addition incorporation cereal crops with legume plants enhanced fixed nitrogen to plants and reduce the need of synthetic fertilizer need (Li et al. 2011). Inter cropping also cause to increases root biomass and expansion, which contributes to soil structure improvement and microbial activity of soils (Zhang et al. 2025).

2.6.1 Comparison of Simple Rotation vs Complex Intercropping Systems

Cropping systems can be categorize according to their diversity and temporal arrangements. Mono-cropping refers to continuous cultivation of single crop in a same land over a successive cultivation seasons. Crop rotation involve cultivation of different crops on a same field over the time. This crop rotation systems offer basic benefits such as improved nutrient cycling and suppression of pest (Rakotomalala et al. 2023). In simple crop rotation, typically consists of two or few crops while complex crop rotation include grater diversity of crops often incorporating legumes,cover-crops or green-manure crops. However by combining plants with different nutrient requirements and with different root structures, cause to improve overall field health and productivity of the production (Brooker et al. 2015). Complex systems increase microbial diversity and improve soil health by providing a wide range of substrates for microorganisms. However, these complex inter-cropping systems require more management, and careful planning. Cost is also high (Zhang et al. 2025). But integrating inter-cropping into crop rotation is an effective approach to improve soil health and overall sustainability in organic greenhouse vegetable production systems. As an example from field crop cultivation, ,cereal and legume inter-cropping shown some data related to increase the community of beneficial arthropods and suppress pest and insects compared with mono cropping systems, thereby it helps to create natural pest controlling in the field production system (Rakotomalala et al. 2023).

2.6.2 Sugar Pea (*Pisum sativum*) in Organic Greenhouse Crop Rotation

Sugar pea (*Pisum sativum*) is an annual herbaceous legume with well developed taproot system and lateral roots which provides root surface for microbial colonies. Above ground part of the plant has compound leaves, tendrils and having a climbing growth habit . The root system of sugar pea plays a vital role for fixing nitrogen by hosting to numerous symbiotic rhizobial bacteria within root nodules (Davis 1985).

Formation of nodules begins at root hair zone, where the rhizobia can infect the plant through root hair by creating infection threads to penetrate cortical cells. Sugar pea typically form indeterminate nodules, which having persistent apical meristem which has the ability of continuous growth and development.

Morphologically, these nodules in pea have distinct internal zonation, called meristematic, infection, nitrogen fixation zone, and senescence zone. The nitrogen fixation zone including differentiated bacteroids that convert atmospheric nitrogen into ammonia using nitrogenase enzymes. This structure cause continuous nitrogen fixation over time (Udvardi & Poole 2013).

Sugar pea (*Pisum sativum*) is a widely used legume crop under organic greenhouse systems, because it has good capacity of rapid growth and nitrogen fixation capability. (Peoples et al. 2009). In greenhouse environments, where intensive cropping systems are common, sugar pea can be integrated between main crops to improve soil fertility (Jensen et al. 2010). The short growth cycle of sugar pea allows for flexible crop rotation scheduling in controlled environments. Due to highly active sugar pea plant rhizosphere, exudates releasing from roots stimulating microbial growth and their activity (Bais et al. 2006). These releasing exudates provide food sources like carbon for microorganisms, cause proliferation of microbial biomass. This helps directly to nutrient cycling and for nutrients availability. For intercropping systems, sugar pea improve nutrient uptake efficiency and increase productivity through its symbiotic association with nitrogen fixing bacteria, that can enhances biological nitrogen availability. However, the benefits of sugar pea are often short term due to its annual growth habit (Philippot et al. 2013; Chen et al. 2023).

2.6.3 Clover (*Trifolium* spp.) in Organic Greenhouse Intercropping Systems

Clover (*Trifolium* spp.) is a perennial legume which has a fibrous expanding root system, which provides multiple locations for nodule formation (Frame et al. 1998). Root structure of clover is advantageous for long term nitrogen fixation from agroecosystems. Clover (*Trifolium* spp.) is also used in organic production for not only as a cover crop but also as living mulch due to its long term influence for soil fertility and sustainable soil management (Thorup-Kristensen 2006). Not

like other annual legumes, clover provides continuous nitrogen supply through biological nitrogen fixation because of its perennial nature (Herridge et al. 2008). Clover improves soil structure through its extensive root system, which enhances soil aggregation and water infiltration (Bronick & Lal 2005). The continuous presence of clover roots provides a stable environment for soil microorganisms. This leads to increased microbial diversity and improved soil health (Lori et al. 2017).

In greenhouse systems, clover is often used as an inter crop or living mulch to maintain healthy soil and it helps to suppress weed growth likewise sugar pea, clover also helps to enhance nutrient cycling and reduce nutrient losses from soil (Snapp et al. 2005). Intercropping main cash crop with clover as nitrogen fixing crop, is also improve soil biological activity by providing continuous organic inputs to crop.

Table 1 Comparison of sugar pea and clover in relation to nitrogen dynamics, soil health, and microbial activity.

Characteristic	Sugar pea (<i>Pisum sativum</i>)	Clover (<i>Trifolium</i> spp.)	References
Growth habit	Annual legume crop	Perennial or biennial legume	(Sorensen & Thorup- Kristensen 2003)
Nitrogen fixation	Rapid nitrogen fixation	Continuous nitrogen fixation	(Sorensen & Thorup- Kristensen 2003)
Nitrogen availability	rapid release of nitrogen residues after incorporation	Gradual nitrogen supply	(Sorensen & Thorup- Kristensen 2003)
Effect on soil structure	Limited long-term effect	Improves soil aggregation through extensive roots	(Sorensen & Thorup- Kristensen 2003)

Purpose and Research Questions

2.7 Background/problem Description:

The European Union (EU) has recently introduced new regulations for organic production. The new regulations state that all organic production must be take place directly in the soil, and a crop rotation is mandatory. Additionally, the crop rotation must include a green manure crop that can add nutrients to the soil. The change in regulations means a big change in production for many organic greenhouse producers in the northern European countries, who has previously had the option of producing in demarcated beds and in pots using different types of growing media. With the short production season in the Nordic countries, producing directly in soil could mean a large risk of introducing soil borne pathogens that can make severe consequences for plant health.

The key objective of this project was to examine the effects of two legume based production systems on soil biological health in an organic production systems. Two different crop systems differed in the type and management of the legume crop, are compared: 1) an annual sugar pea (*Pisum sativum*) grown as a green manure crop within a crop rotation and 2) a perennial clover (*Trifolium repens*) established as a living mulch intercropped with cucumber. Soil samples are taken after each crop in the production to assess changes in selected biological soil health indicators.

2.8 Aim and Research Questions:

To compare the effects of two different crop rotation systems, an annual sugar pea green manure rotation and a perennial clover on biological soil health in organic greenhouse production.

How do different legume based cropping systems influence biological soil health in organic greenhouse vegetable production?

2.9 Hypothesis:

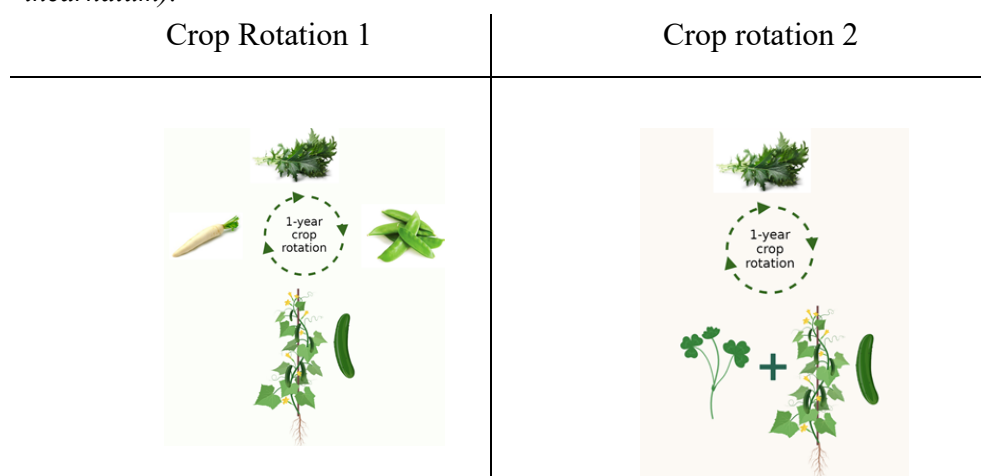
Crop rotation complexity with inter-cropped legume green manure, improves overall biological soil health as indicated by soil respiration, protein content, available carbon, available nutrients and microbial diversity of the soil.

3. Materials and Methods

3.1 Experimental Set-up

This greenhouse experiment was conducted at Mellongård, Lomma which has been used to practice organic production for many years. Crop rotation was practiced inside two individual neighbouring greenhouses. Both are plastic poly tunnels with no special systems for light and heat control. This study is an experiment that basically examining how crop rotation complexity with intercropped legume green manure, contribute to improves overall biological soil health in organic greenhouse production. And also, through this experiment, compares the microbial soil health of two different crop rotation systems for year around. One is crop rotation with sugar pea. (*Pisum sativum* var. *saccharatum*) Other one is intercropping cucumber with crimson clover (*Trifolium incarnatum*).

Table 2 ; Two crop rotation systems, Rotation 1 crop rotation with Mizuna leafy greens, Sugar pea, Cucumber and Daikon raddish.. (*Pisum sativum* var. *saccharatum*), Rotation 2 Mizuna leafy greens and intercropping cucumber with crimson clover (*Trifolium incarnatum*).



By using these two crop rotation systems, microbial soil health Microbial soil health was determined by using five different soil health indicators. Those were microbial activity, soil respiration, soil protein, soil active carbon, soil organic matter and available nutrient analysis. All the laboratory analysis was done in Bio-safety level (BSL) 2 laboratory which locate at Department of Bio-systems and Technology belongs to the SLU campus Alnarp. Each greenhouse production area was divided into three beds (Three blocks) and each bed was divided into two plots. Each greenhouse consisted of three plots per each treatment. Each plot was named with letters. (A to L). These blocks were created 6 replicates for each

rotation as shown below. In here sugar pea succeed by cucumber represented as rotation 1, R1 and clover inter-cropped with cucumber represented as rotation 2, R2. (Figure 11)

3.2 Soil Sampling

Table 3 ;Soil Sampling Dates for sample round 4,5,6,7 and 8

Sample	Sampling Date
Sample 4	2024 December
Sample 5	2025 April
Sample 6	2025 June
Sample 7	2025 September
Sample 8	2026 March

Each greenhouse consists with six sampling plots. Soil samples were then collected using a soil auger with 30 cm column. From each plot, eight sub samples were taken randomly to ensure representative sampling of the soil within the block. After that, the eight samples from each block were put together and mixed to get uniform soil sample, and then samples were transferred into separate, clean, closable plastic bags. Then the samples were labeled with the relevant letter of the block. Finally, all soil samples were stored in cool room (5-8°C) to preserve their physical and chemical properties until laboratory analyses were conducted. (Moebius-Clune 2016)

3.3 Laboratory Analysis

Before each laboratory analysis, soil samples were removed from the refrigerator and air-dried overnight. The samples were then sieved using a 2 mm sieve.

3.3.1 Soil Active Carbon Analysis

Soil active carbon analysis was done by based on CASH protocol . First 0.02 M KMnO_4 solution was made using CASH protocol procedure. After preparing the solution, bottle was covered with aluminum foil. Because this solution sensitive to light. 2.5g of air- dried sieved soil were measured and added into the 50ml falcon tubes. Then the falcon tubes were covered with aluminum foils. Then 18 ml of distilled water and 2 ml of the 0.02 M KMnO_4 solution to the tubes was added to start redox reaction. Then the tubes were shaken on a shaker for 2 min at 120 rpm. After the 2 min the tubes were removed from the shaker. Afterward samples were kept on bench top to settle 8 minutes. After 10 minutes of total reaction time, 0.5ml of sample was added into new falcon tube set which containing, 49.5ml of

water. Then all the tubes were shaken by hand for 10 s. Finally transferring 1 ml of the sample to the cuvette and the samples were measured in a spectrometer at 550nm. To interpret the data a standard curve was created by making a standard dilution series of KMnO_4 . By using CASH formula active carbon content was calculated (Moebius-Clune 2016).

3.3.2 Soil Protein

Soil protein was analyzed using CASH protocol (Moebius-Clune 2016). First soil samples were air dried overnight and sieved by using 2mm sieve. Then three grams of soil was weighed out and transferred into 100ml reagent bottles. After that 24ml of 20mM sodium citrate with pH 7 were added into reagent bottles with soil with using 10 ml pipette and pipette boy. Afterward bottles were capped tightly and Shaked by using shaker (0025002982, HS 501 D, IKA, Staufen im Breisgau, Germany) for 180rpm, 5 min. Subsequently the bottles were swirled. After these bottles with solutions were autoclaved for 30 min in 121 and 15psi. Before autoclaved bottle caps were loosened to avoid the buildup of unnecessary pressure. After autoclaved, samples were kept until become room temperature. Then 1.75ml of the slurry was withdrawn with avoiding soil lipid layer to small micro centrifuge tubes. Then the tubes were centrifuged at 10,000rpm around 3 min to remove soil particles. 10 μ l of the sample and standards (23209, Albumin Standards, Thermoscientific, Rockford,IL, USA) were transferred into 96 wells micropipette plate and 200 μ l of BCA (Bicinchoninic Acid) working reagent (23227,Pierce BCA Protein Assay Kit, Thermo scientific, Rockford, IL, USA) were added into all wells including standards. After that plate were sealed and put into water bath for 60 min at 61.5 $^{\circ}$ C. After the incubation, sample was cooled for 10 min. Then the Absorbance was read by using spectrophotometer (A51119700, Mulitskan SkyHigh, Thermoscientific, Singapore) at 562nm. Finally soil protein was calculated by using standard protocol (Moebius-Clune 2016).

3.3.3 Soil Microbial Activity

Anlysis of fluorescein di-acetate activity (FDA) method was used to measure soil microbial activity Green et al. 2006). Aliquotes of 1g of air-dried soil was placed in a 100 reagent bottles. 50 ml of sodium phosphate buffer and 0.5 ml of 60mM FDA lipase substrate solution were added into reagent bottles with soil samples. Subsequently the lids were tight and swirled few minutes to mix the contents. Then samples were kept in incubator for 3h at 37 $^{\circ}$ C.

After three hours of incubation, 2ml of reagent grade acetone was added into the suspension and mixed it well to terminate the reaction of FDA hydrolysis. After mixing, approximately 35 ml of solution was transferred into other 50ml centrifuge tubes and centrifuge for 8000rpm, 5 minutes. Then the centrifuged

solution was filtered using Whatman No 2-filter paper (CAT No.1001-150, Cytiva, Shanghai, China). Finally from that solution 1 ml was transferred into cuvette using micropipette. Finally using spectrophotometer (LPV440.99.00001, DR3900, Hach, Düsseldorf, Germany), the solution absorbance was measured at 490nm. (In here controlled sample was also maintained with adding 0.5 ml of reagent grade acetone instead of adding FDA lipase solution.)

3.3.4 Soil Respiration

Soil respiration was measured using CASH protocol. Wide opening 500ml glass jar bottles with lids were used to this analysis. Twenty gram of overnight air dried soil were put into aluminium weighing boat. Before adding soil, 9 small holes were pierced in bottom of the each aluminium boat. Then glass jar bottle bottom was covered with 2 filter papers (2 Whatman filter papers with 7 cm in diameters). Then aluminium boat with soil was placed on top of the filter paper as touch the bottom holes of the aluminium boat. Tripod like stand was constructed by using chicken wire and plastic lid. It was placed on top of the soil boat. On top of the plastic lid, 10 ml glass beaker was placed and tapped. Aliquots of 9 ml of 0.5 M potassium hydroxide (KOH) solution was added into beaker to act as CO₂ trapping solution. To hydrate the filter papers soil 7 ml ddH₂O was added to the filter paper by using pipette. A blank was made the same way as the samples without soil. This was done to measure the original amount of CO₂ that was trapped in the jar at the start of the analysis. After adding water and KOH into construction, the jar lid was closed securely with the screw top lids making them airtight and they were incubated for four days (around 96 h) in fume hood at normal room temperature. After four days of incubation the jars were opened, and the electrical conductivity of the KOH was measured using a electrical conductivity meter (HQ440D.98.00012, HQ440d multi conductivity meter, Hach, Düsseldorf, Germany). The soil respiration was measured by calculating the amount of CO₂. Finally the values were then compared with the conductivity of pure 0.5 M KOH solution, which simulates a fully saturated trap solution of KOH.

3.3.5 Soil Organic Matter Analysis

For analysis total organic matter content of soil, the loss through gnition was used. Initially, collected soil samples were sieved and air dried. 35 g of the air dried soil were measured into pre cleaned aluminum containers. Then the air dried samples were first oven dried at 105 °C for 24 h to remove all moisture. Then samples were weighed. Subsequently, the oven dried soil samples were transferred into heat resistant porcelain pots which capable of withstanding heat. The samples were then kept inside muffle furnace (Model: CEWE BAS 63 ABB, Sweden) at 550 °C for 2 h This high temperature ignition process oxidized and removed organic

matter as carbon dioxide. Then burned samples were weighed. Finally by using before and after burned values, organic matter percentage was calculated. LOI (Loss of Ignition) method was used to calculate the organic matter percentage (Moebius-Clune 2016).

3.3.6 Soil Available Nutrients

A standard analysis of soil available nutrient was measured by using Spurway analysis. This was done by the company LMI AB located in Helsingborg, Sweden. The nutrient analysis comprised the amounts of total nitrate, ammonium, phosphorus, potassium, magnesium, sulphur, calcium, manganese, boron, iron, sodium and aluminium. In addition electrical conductivity and pH were measured.

3.4 Data Analysis

All response variables were analyzed separately using linear mixed-effects models. The analysis included sample, rotation, and their interaction as fixed effects. Random effects were specified to account for the block and plot structure of the experiment. Specifically, plot was nested within bed, and an additional random term was included for the sample-by-bed combination. The model used for the analysis was:

$$Y_{ijkl} = \mu + S_i + R_j + (S \times R)_{ij} + B_k + P_{l(k)} + (S \times B)_{ik} + \epsilon_{ijkl}$$

Where:

Y_{ijkl} = observed value of the response variable,

μ = overall mean

S_i = Sample effect (Fixed effect), where sample represents sampling time

R_j = Rotation effect (Fixed effect)

B_k = Random effect of the k^{th} bed, where bed represents the six units formed from two greenhouses and three blocks per greenhouse,

$P_{l(k)}$ = Random effect of the l^{th} plot nested within the k^{th} bed, where plot represents the bed part or treatment unit within each bed,

$(S \times B)_{ik}$ = Random effect of the interaction between sample and bed, included to account for bed-level variation across sampling times,

ϵ_{ijkl} = residual error

In R notation, the fitted model was:

`lmer(response ~ sample * rotation + (1 | bed/plot) + (1 | sample:bed), data =`

The model was fitted using restricted maximum likelihood (REML) estimation. Type III analysis of variance (ANOVA) with the Satterthwaite approximation was used to assess the significance of fixed effects. Estimated marginal means were

obtained using the emmeans procedure, and pairwise comparisons were performed using Tukey's adjustment at the 5% probability level. Statistical analyses were conducted using R version 4.6 within the RStudio environment version 2026.04.0-526. Mixed-effects models were fitted using the lmerTest package, while estimated marginal means and mean comparisons were generated using the emmeans package.

4. Results

4.1 Analysis of Variance for Soil Health Indicators

Table 4; Results of the Analysis of Variance (ANOVA) showing the effects of sampling time, cropping systems and their interactions on biological and chemical soil health indicators.

Parameter	Sampling time	Cropping system	Sampling time* Cropping system
Soil active carbon	4.98**	1.99	0.17
Soil protein	24.44**	0.03	1.99
Organic matter Percentage	8.60**	1.90	0.87
Ph	66.20**	2.58	6.51**
Electrical conductivity	33.90**	4.02	4.64**
Nitrogen	35.19**	1.37	4.31**
Phosphorus	4.98**	0.23	0.92
Potassium	1.05	11.48*	1.10
Amount of fluorescein	77.78**	0.04	0.24
Soil respiration	1.96	0.00	3.74*

* for Significant at 0.05 and ** for Significant at 0.01.

According to the ANOVA results, sampling date was the major factor affecting most soil health indicators, showing higher effects on active carbon, soil protein, organic matter, microbial activity, pH, EC, nitrogen, and phosphorus. Moreover crop rotation had limited effects and was significant only for potassium. Significant interaction effects between sampling date and crop rotation were observed for pH, EC, nitrogen, and soil respiration, indicating that the response of these parameters to crop rotation varied over time.

4.1.1 Soil active carbon

Considering the soil active carbon graphs, showed a significant effect of sampling time ($p < 0.01$). Crop rotation and sampling rotation interaction effect was not significant.

Active carbon values remained almost stable across two rotation treatments, with the highest value observed in September 2025 under R2 and the lowest recorded

in December 2024 under R1. The value obtained as 909.39 mol/l and the lowest active carbon value was recorded in 2024 December with R1 as 617.04 mol/l. Considering the overall values, soil active carbon remained relatively stable among sampling dates and rotations treatment. This results of soil active carbon indicate that soil active carbon content remained relatively stable across most of sampling dates and rotation combinations, with only limited treatment specific variations can be observed.

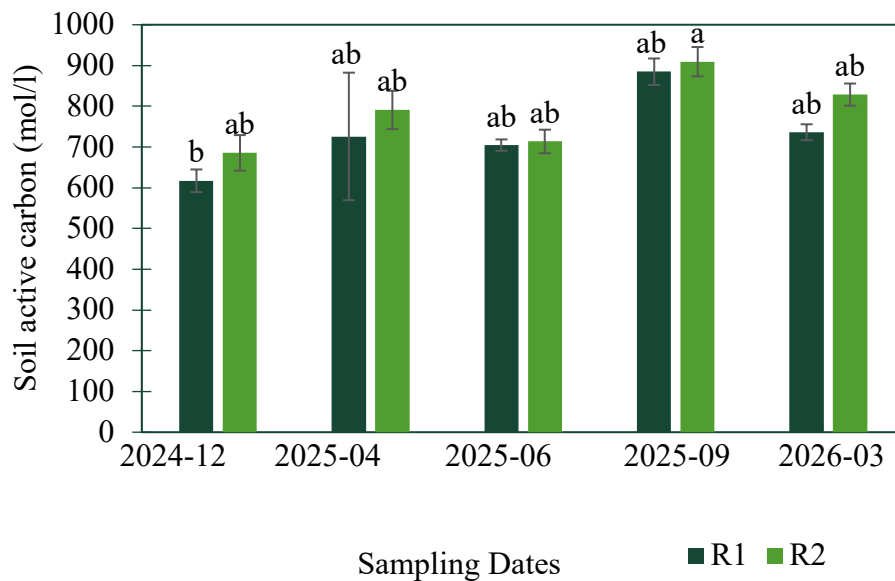


Figure 1 ; Effect of sampling time on Soil active carbon. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

4.1.2 Soil protein

Considering the soil protein graphs, showed a significant effect of sampling time ($P < 0.01$). Meanwhile crop rotation and sampling rotation interaction effect was not significant. This means that biological available nitrogen fraction were basically depend on temporal changes, not with crop rotation. The highest soil protein value was recorded in 2024 December under R1. The highest soil protein content recorded as 11.45 mg/g soil. Meantime, the lowest value was observed in 2025 April under R2 as 7.53 mg/g soil. Considering the sampling dates and rotations, significant differences were mainly observed among different sampling occasions rather than between rotations.

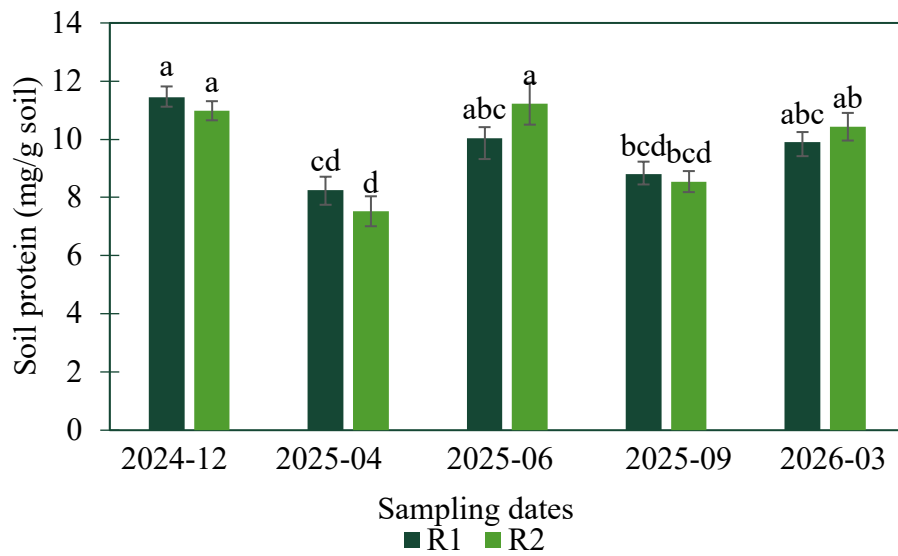


Figure 2 ; Effect of sampling time on Soil protein content . Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

4.1.3 Organic Matter Content

Considering the result of soil organic matter content, shows a significant effect with the sampling time ($P < 0.01$), with no significant effects when compared with rotation and also interaction.

The highest organic matter percentage was observed as 4.30% on 2025 September under R, while lowest value was recorded in 2025 April as 2.80% under R2. Then organic matter content ranged between 2.80% and 4.30%. These values indicate moderate but stable organic matter levels typical for intensively managed greenhouse soils.

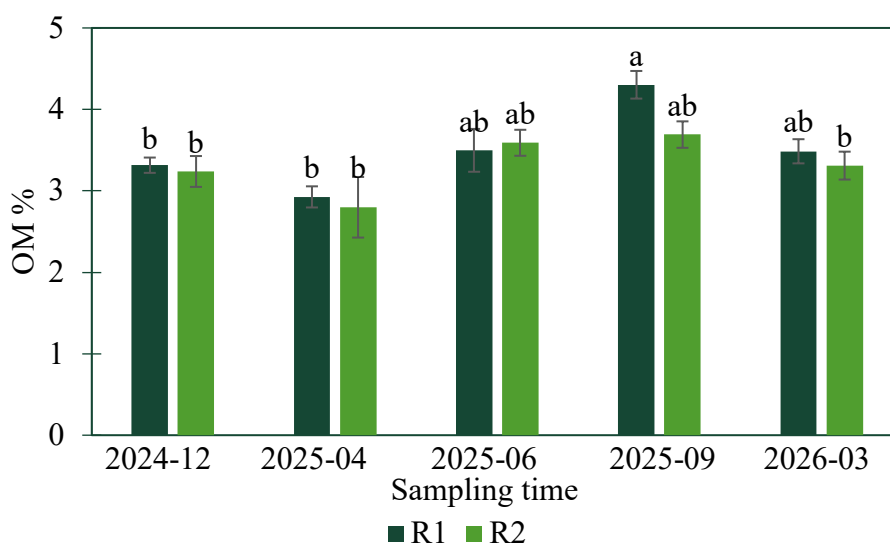


Figure 3 ; Soil organic matter content variation among different soil sampling periods.. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

4.1.4 Soil Microbial Activity

Soil microbial activity, measured by using fluorescein diacetate (FDA) hydrolysis analysis, showed a significant differences when considering sampling time ($P < 0.01$), and the meantime no significant difference of rotation or interaction was observed considering ANOVA test values. This indicates that microbial enzymatic activity change time. There was not not direct influence from different crop rotations.

Higher microbial activity was observed in June 2025 and it was 243.09 mg/kg, whereas lowest values were observed in March 2026 and value was 130.39 mg/kg. (Figure)According to values can suggest that seasonal variations can be cause to this microbial functioning.

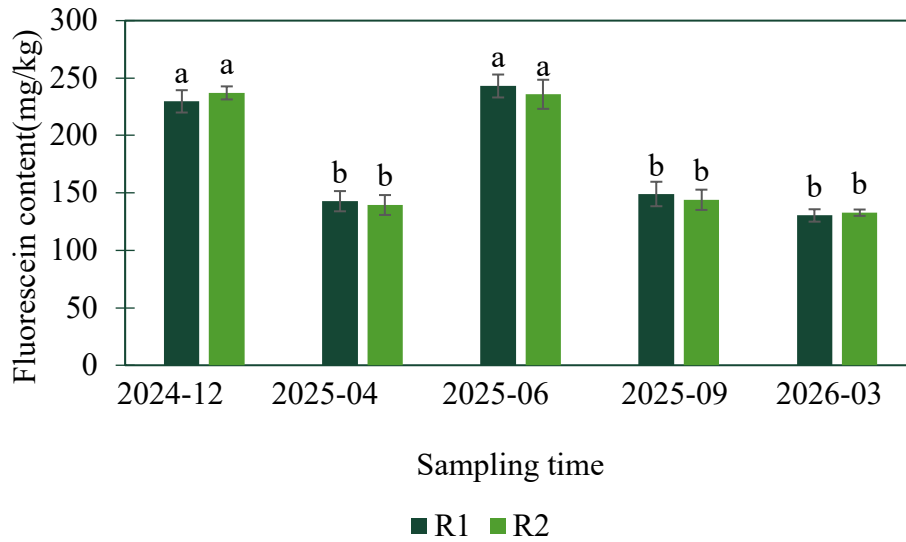


Figure 4; Effect of sampling time on Soil microbial activity . Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

4.1.5 Soil respiration

Soil respiration showed a significant effect between sampling time and crop rotation ($P < 0.05$), while no significant main effects were detected neither sampling time nor between rotations This indicates that the effect of crop rotation on microbial respiration depended on the sampling period.

Higher respiration rates were observed in later sampling periods (2025 September and 2026 March), particularly under R1, suggesting increased microbial activity under specific temporal conditions. In contrast, lower or even negative values observed in earlier sampling periods may indicate reduced microbial activity or measurement variability under low substrate conditions. The greatest soil respiration occurred during 2026 March with a value of 0.08 mg, while the lowest soil respiration in R1 occurred during 2025 June with a value of -0.04 mg

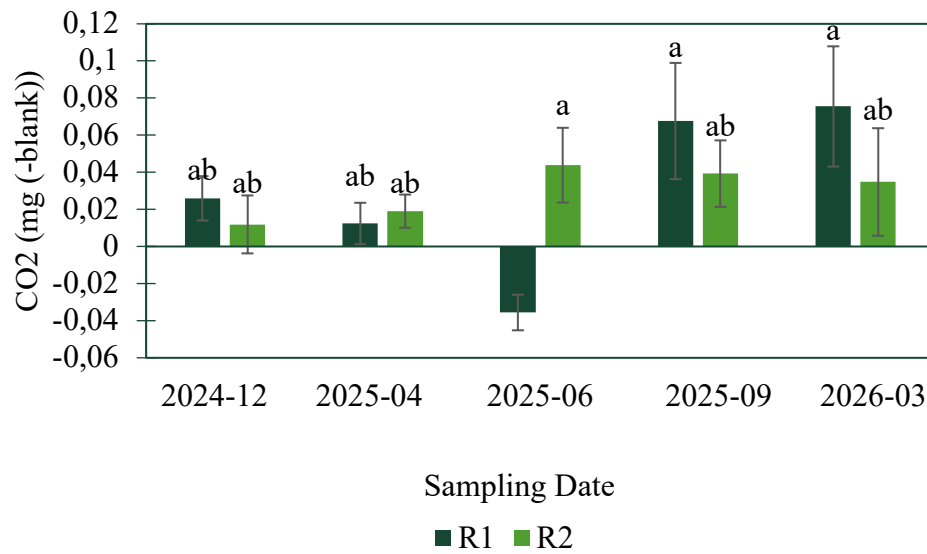


Figure 5; Soil respiration variation among different sampling times. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

4.1.6 pH

Considering soil pH values, there were significant effects between sampling time ($P < 0.01$), and also significant effect between sampling time and rotation interaction ($P < 0.01$). But there was not significant effect between rotations. This indicates that pH changes can be directly change with management system and with time. The pH range between 6.30 to 6.80, and this range within the optimal range basically for greenhouse vegetable production.

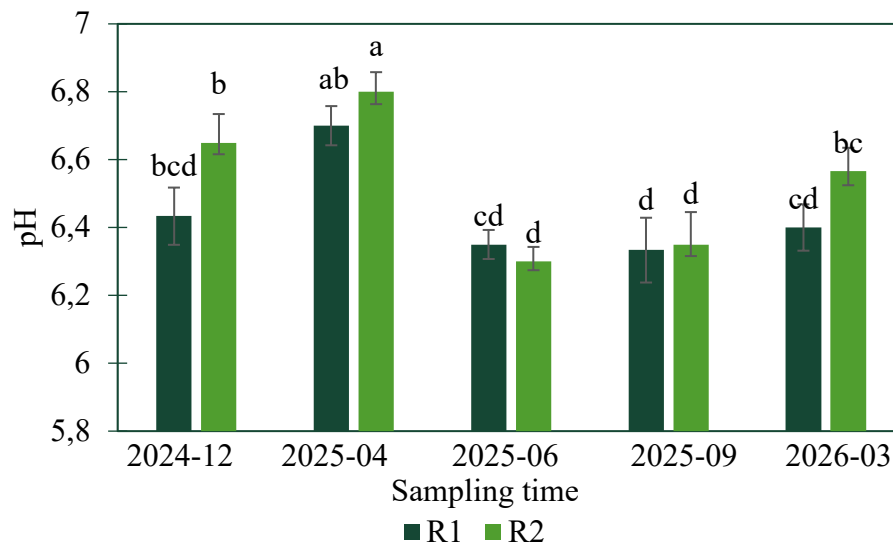


Figure 6; Soil pH variation among soil sampling dates. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

4.1.7 Electrical conductivity

Electrical conductivity showed significant effects of sampling time and rotation ($P < 0.05$), as well as a significant interaction. This indicates that salinity levels were influenced by both temporal changes and crop rotation system, with rotation effects varying across sampling periods.

The highest electrical conductivity was observed in 2025 June under R2 (0.79 mSm), and the lowest value was recorded in 2025 April under R2 (0.27 mSm).

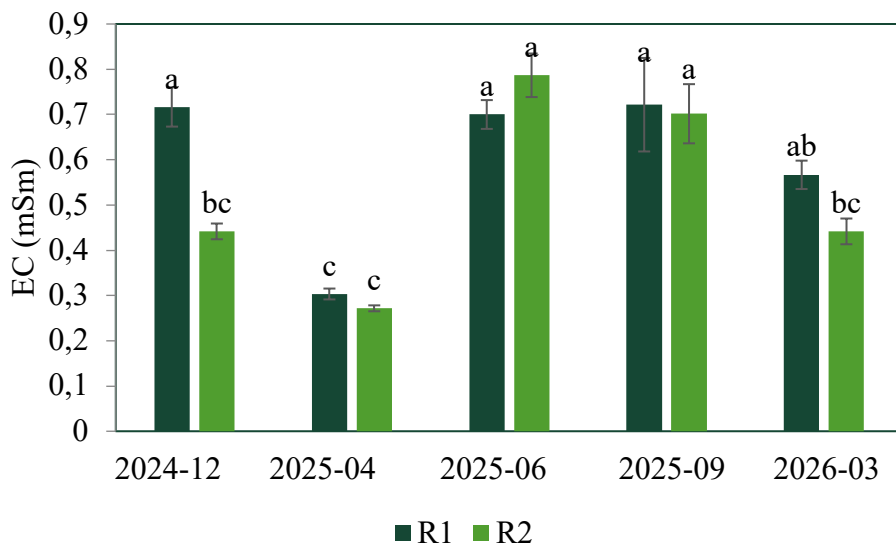


Figure 7; Soil EC variation among soil sampling dates . Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

4.1.8 Plant nutrient

Nitrogen

Soil nitrogen showed significant effects of sampling time ($P < 0.01$) and a significant interaction effect ($P < 0.05$), while the main effect of rotation was not significant. This indicates that nitrogen availability was directly related only with time and not with different rotations.

The highest nitrogen content was recorded in December 2024 under R1 and value was 32.50 mg/l. whereas the lowest value was recorded in April 2025 under R2 and value was 4.62 mg/l. There were significant differences between rotations were observed only at December 2024.

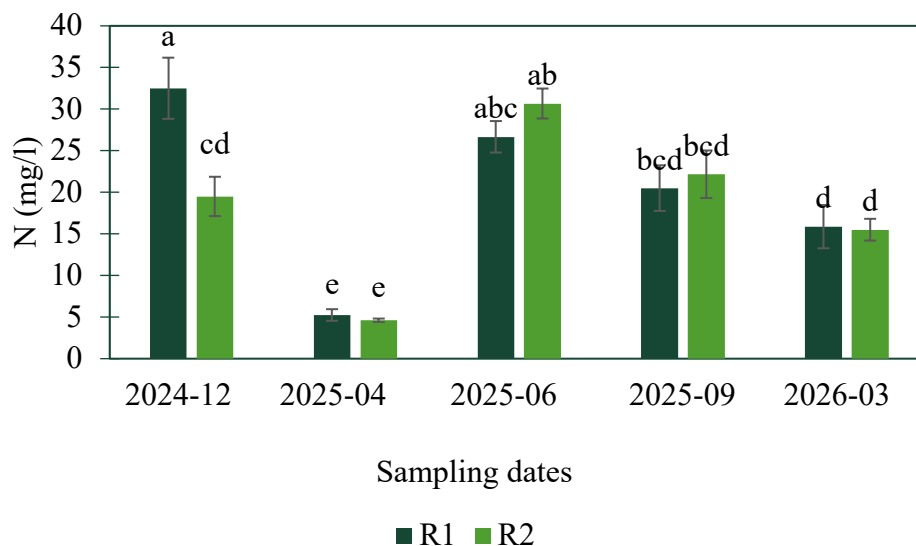


Figure 8; Soil nitrogen concentration variation among soil sampling dates. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

Phosphorus

Considering phosphorus content it shows significant difference within sampling time ($P < 0.05$). There were no any significant rotation or interaction effects according to the p values which got from ANOVA test. This might be due to that phosphorus availability was stable across rotation systems and mainly effected by temporal variation. The highest phosphorus value was recorded in 2025 June under R1 (21.17 mg/l), while the lowest value was observed in 2025 April and value was R2 (15.83 mg/l).

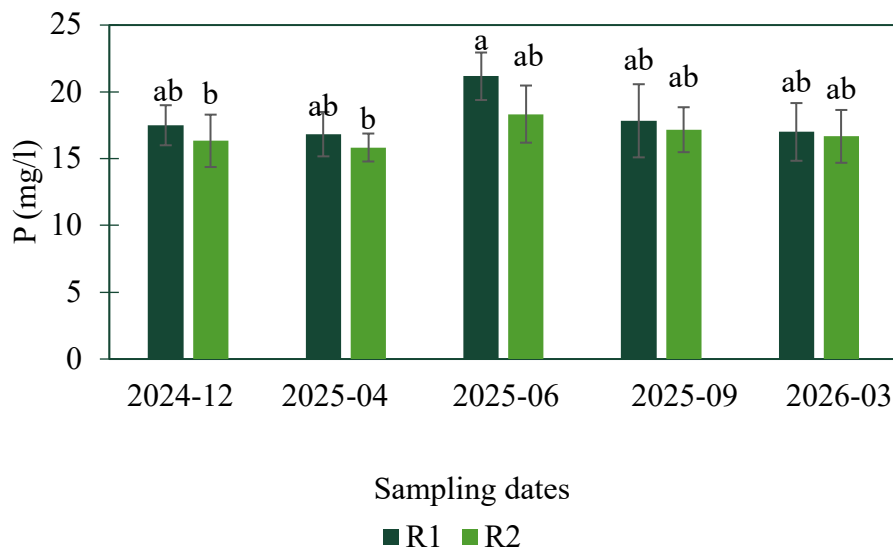


Figure 9; Effect of sampling time on soil phosphorous concentration (mg/l). Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

Potassium

Potassium content showed significant rotational differences at 2024 December and 2026 March, where R1 values were higher than R2. The highest potassium value was observed in 2024 December under R1 (31.67 mg/l), while the lowest value was recorded in 2025 April under R2 (20.67 mg/l).

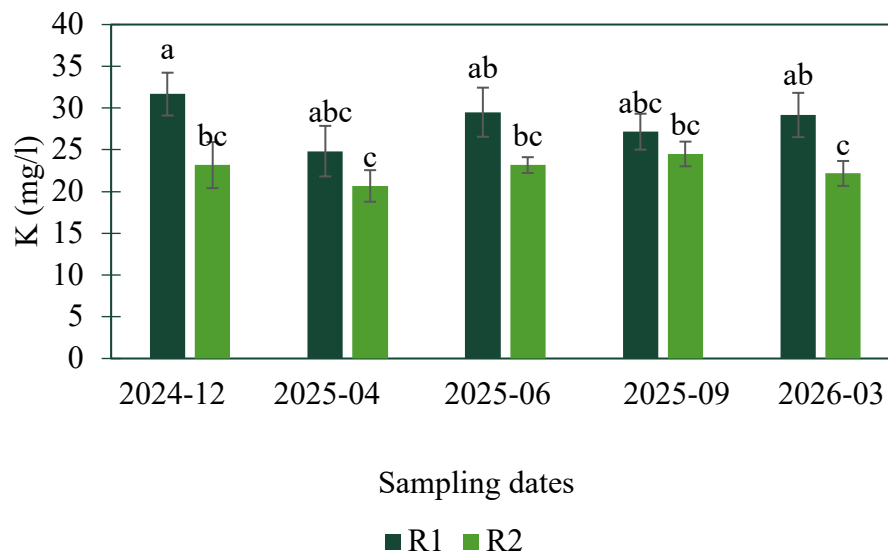


Figure 10 ;Effect of sampling time on soil potassium concentration (mg/l). Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

5. Discussion

This study basically evaluated the influence of two different crop rotation systems, an annual sugar pea green manure rotation and a perennial clover on biological soil health in organic greenhouse vegetable production under soil based cultivation system. In here two crop rotation systems were compared. One was with sugar pea based rotation system and second one was with inter cropping system with cucumber and clover. Considering the overall impact of the research, the findings demonstrated that, different sampling time had stronger influence on most of the soil indicators than the different crop rotation systems. But there were some interaction effect between sampling time and rotation.

The research hypothesis was increased crop rotation complexity with intercropped legume green manure, improves overall biological soil health as indicated by soil respiration, protein content, available carbon, available nutrients and microbial diversity of the soil.

However the results not support on this hypothesis and hypothesis was rejected according to results. This results suggest that all soil health parameters change over time and, not with different rotation systems with this experimental period also according to the Lori (2017), soil microbial responses in organic systems are mostly influenced by environmental and seasonal factors rather than crop rotations (Lori et al. 2017).

Soil active carbon showed significant differences among sampling dates according to the ANOVA table while crop rotation and their interaction were not significant. Active carbon considered as most sensitive parameter of labile organic matter and microbial activity. It directly reflects the readily decomposed carbon fraction from root exudates, plant residues and from turnover of microbial biomass (Moebius-Clune 2016). Therefore the observed temporal fluctuation can be due to changes of crop growth stage, decomposition process and different temperature and soil moisture conditions.

Higher active carbon amounts observed in later sampling periods. It may be due to higher microbial activity on organic inputs and can be due to increased rhizosphere activity. According to similar finding from Culman et al.,(2012), active carbon responses are higher to biological And seasonal changes in agricultural soils. In this present study absence of significant difference between two crop rotations may indicate sugar pea and clover provided more or less similar carbon input to soil microbial communities. Moreover, this duration of the experiment may not have been enough to observe reliable rotational effect especially on soil carbon dynamics (Culman et al. 2012).

When considering soil protein content, It also varied significantly among sampling dates. According to P values rotational effects were not significant. Soil protein is an important indicator. It measures specially organically bound nitrogen and microbial residue accumulation in soil (Moebius-Clune 2016). Hence soil protein variation might be directly influenced by microbial activity, decomposition rates and mineralization of nitrogen in cropping cycle.

Higher soil protein concentrations can be observed during the sampling period of 2024-12 and 2025-06 associated with high soil microbial activity, cause to microbial biomass turnover and increased organic nitrogen availability by residual decomposition. Legume crops are generally contribute to the soil nitrogen through symbiotic fixing of nitrogen (Peoples et al. 2009). Legume contribution to the soil nitrogen mainly depend on the balance between nitrogen input though fixing of the biological nitrogen and fertilizer application and also nitrogen removal through plant uptake. But more precise effects can be observed under long term management (Peoples et al. 2009).

Soil organic matter plays major role specially in maintain soil structure, nutrient retention, microbial habitat stability and water retention capacity (Bronick & Lal 2005). In this study relatively stable organic matter percentage suggested that both greenhouse system maintain under moderate soil fertility condition except seasonal fluctuations.

2025-06,2025-09 and 2026-03 sampling periods showed higher organic matter percentages indicated that high accumulation of organic crop residues. The absence of clear rotational differences may be due to measurable changes in soil organic matter require long term implementation for cropping system with crop diversity.

Soil microbial activity measured using FDA hydrolysis visualized significant difference among some sampling time but not between rotations. FDA hydrolysis basically indicate the total enzymatic activity of the microorganisms and it commonly used as a good indicator for overall soil microbial activity and functioning (Green et al. 2006). According to the significant value can suggested that soil microbial activity varied mainly with time. Not depend on crop rotational variation. In this experiment higher microbial activity can be observed in 2025-06 and and 2024-12 indicated the occurring warmer temperature not the only reason for higher microbial activity. High microbial activity can be a result of crop residue decomposition under warmer temperatures. Root exudates released during active plant growth can stimulate microbial metabolism and cycling of nutrients within rhizosphere (Bais et al. 2006)

Considering soil respiration, It is strongly influenced by soil organic matter content, soil temperature, soil aeration, moisture, and available microbial biomass (Franzluebbers et al. 2000b). There is close relationship between soil respiration and soil organic matter content. Increased carbon substrate availability directly stimulated microbial metabolism. Similar findings observed that there is a positive relationships between soil organic matter and microbial respiration in intensively managed agricultural soils (Creamer et al. 2014)

The lower respiration may be due to less microbial activity or can be due to substrate limitation. Negative or low respiration values can be due to low biological activity, or less microbial efficiency under conditions such as limited aeration or imbalance of nutrients (Moebius-Clune 2016). Negative respiration values might be resulted from methodological sensitivity limitations under low CO₂ production conditions. Control-corrected respiration values occasionally can produced negative results, indicating lower microbial respiration relative to the control treatment

Management practices like adding crop residues, reduced soil disturbance, and maintenance of suitable soil moisture can improve soil respiration and biological activity in greenhouse soils. Organic amendments provide energy for microorganisms and it improve improve soil aggregation, then it enhance microbial activity (Agegehu et al. 2017).

Macro nutrients such as nitrogen, phosphorus, and potassium are vital elements for the proper functioning of crops. As for this study, there was significant variation in soil nitrogen and potassium content in different samples and some of the tested rotations while phosphorus showed no significant difference between treatments. The possible reason for different nitrogen values is the variability in organic matter decomposition, and nitrogen mineralization in the greenhouse soil. Nitrogen fixing crops also directly effect on the nitrogen values. Higher potassium levels were identified in many R1 rotations than in R2 rotations, implying that rotation has an effect on that. Moreover, phosphorus was consistent throughout the different treatments, suggesting equal phosphorus availability across the experimental soils. The obtained data demonstrated reasonable nutrient concentrations within the recommended ranges for growing greenhouse crops that require adequate N, P, and K levels for healthy root development, photosynthesis process, flowering, fruit development, etc (Havlin 2014). Moreover repeated fertilizer application under greenhouse condition can cause increasing of salinity issues. Because there is no rainfall inside the greenhouses and leaching is limited. This kind of nutrient accumulations have been reported as a major challenge in soil based greenhouse production (Havlin 2014). Nitrogen showed major differences across sampling dates and showed interaction effects from sampling time and rotation. Within soil systems, nitrogen is the most variable and most influenced by a combination of mineralization, microbial immobilization, plant

uptake, and situational effects (Havlin 2014). The early sampling periods having a higher value of nitrogen could be a result of recently occurred mineralization of the organic residue, coupled with legume crops' nitrogen fixation.

Although continuous inputs of nitrogen through biological nitrogen fixation were expected due to clover intercropping, strong long-term rotational effects on soil nitrogen were not identified. This finding may be an indication that short-term greenhouse management conditions were more significant in influencing the availability of soil nitrogen than crop diversification. Jensen et al. (2010) reported comparable results, indicating that the benefits of nitrogen resulting from the inclusion of legumes depend on the management level of the environment and the crops (Jensen et al. 2010).

In general, the variability of phosphorus is lower than that of nitrogen, since the capacity of phosphorus is highly dependent on soil pH and the chemistry of the soil (Havlin 2014). As for potassium, notable differences in the levels measured in different rotations were observed on particular sampling occasions (December 2024 and March 2026). These differences may be due to variability in the uptake of the crops and the decomposition of the crop residues.

Variation in soil pH showed the most notable temporal interactions, although the primary crop rotation effect was negligible. The pH remained within the optimal range throughout the study. The values were within the range of 6.30 – 6.80, which is considered to be within the optimum level recommended for greenhouse vegetable production. Variation in pH is often associated with shifts in nutrient uptake as well as microbial transformations of nitrogen, decomposition, and management of irrigation. Fluctuations of pH in greenhouse soils can be primarily attributed to the processes of mineralization of organic matter as well as the activity of soil microbes (Brady et al. 2008).

The soil pH is an important parameter influencing nutrients availability, microbial activities, and soil fertility status. The optimum level of slightly acidic or neutral soils (pH 6.0–7.0) favors nutrient availability and microbial activities (Brady et al. 2008). Proper maintenance of soil pH is critical because both high and low soil pH may limit nutrient availability.

Electrical conductivity (EC) was influenced by significant effects of sampling time, crop rotation, and their interactions. Electrical conductivity (EC) is one of the most important parameters measuring the amount of dissolved salts in the soil and is commonly employed to determine salinity status in greenhouse production systems. In the current study, the overall effect of sample and rotation was significant on EC ($P < 0.05$), which indicated that different samples and rotations had different levels of salinity. . The elevated EC values recorded for some sampling occasions may be an indication of nutrient buildup that is associated with the processes of organic matter decomposition and the subsequent release of

nutrients. In general, the EC values fell within the acceptable limits for greenhouse production, and no significant salinity problems were indicated.

However, too high salinity can decrease water absorption and limit crop production in greenhouse systems (Gruda, 2009). Electrical conductivity (EC) between 1.0 and 2.5 dS m⁻¹ is generally favourable for plant growth. Above 4 dS m⁻¹ are normally associated with salinity stress that can negatively effect on nutrient uptake and plant development (Machado & Serralheiro 2017). However interpretation of EC can be vary with the cultivation system and the growing media. The electrical conductivity levels found in the current study within relatively acceptable ranges for growing vegetables in greenhouses, implying that there were no serious salinity issues in the greenhouse soils.

This study is especially valuable considering the new European Union regulations regarding organic greenhouse production. The regulations now require greenhouse soil-based production accompanied by mandatory crop rotations with incorporated green manure crops. Results show that, although crop rotation complexity had minimal impact on short-term biological factors, the two legume-based systems helped sustain soil health conditions for the duration of the experiment.

The results indicate that variation of environmental conditions, the management of the greenhouse, and the stages of crop growth may play more important roles in controlling soil biological processes compared to short-term rotational complexity. Hence, in order for greenhouse systems to improve biological soil health, the long-term practice of diversified crop rotations may be necessary.

5.1.1 Limitations and Future Research

Some limitations might be considered when interpreting the results of this study.

The experiment conducted over short term period of time and included only two crop rotation systems under greenhouse conditions. But it is necessary to evaluate and compare performance of other legume crops as well. It helps to identify the best legume crop as N fixing and as cover crop.

Inside the greenhouse environmental factors such as greenhouse temperature, humidity, light, irrigation and previous soil management history may also have effect on soil biological responses. Therefore maintain and monitoring inside environmental factors also important.

Additionally, microbial composition of the soils was not measured using molecular methods such as DNA sequencing. Those techniques could provide more scientific understanding of microbial diversity and functional changes within the soil ecosystem.

Future studies should more focus on evaluating long term rotational effects, include additional greenhouse systems, and evaluate microbial community

dynamics using advanced technologies. Because long term influence on soil health is higher according to above findings.

5.1.2 Conclusion of Discussion

- Temporal variation is the main effect on soil health than crop rotation complexity.
- Most of soil properties tend to change with different seasons.
- Legumes helped maintain sustainable, fertile soil conditions.
- Complex rotations showed no clear soil health benefits.
- Long term crop rotations required to improve greenhouse soil health.

According to these overall experiment results, the main findings show that changes over time had an impact on soil health than how complex the crop rotation was in organic greenhouse farming systems. Most soil parameters were significantly affected by when the samples were taken. The effects of crop rotation were generally limited. Legume-based rotations helped keep soil conditions stable. However the benefits of complex crop rotations were not strongly seen during only this period experiment. These findings suggest that implementing crop rotations for a long time may be needed to achieve measurable improvements in soil biological health under greenhouse conditions. The crop rotation complexity did not have an impact on soil health indicators. Soil health indicators were more affected by time variation. The experiments results show that temporal variation had an influence on soil health. Organic greenhouse production systems need term diversified crop rotations. The findings also suggest that crop rotation complexity in greenhouse production systems is not very effective. The temporal variation was a factor in most of the soil health indicators.

5.1.3 Practical Implications

Based on the outcomes of this experiment, we can conclude that all crop rotation systems considered in the study helped sustain biological soil health within an organic greenhouse. Because the majority of soil health parameters showed greater variability due to sampling time compared to the complexity of crop rotations, producers should constantly analyze soil properties over time instead of focusing only on crop rotations.

Moreover, this findings showed that complexity in crop rotation does not result in rapid development of soil biological health in organic greenhouses. Therefore, maintaining consistency in the management practices of soil, such as the use of

legumes, organic matter, and crop rotation, remains vital for the preservation of biological properties of the soil.

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Popular Science Summary

Soil health is a key component of sustainable food production. Apart of proper nutrition of human beings, healthy soil is a key requirement for healthy plants to produce food. In organic agriculture, there are no synthetic fertilizers or chemical pest control measures used. Therefore the maintenance of soil fertility directly depend on natural processes.

The Organic Regulation (EU) 2018/848 of the European Union forced new rule for the maintenance of soil life and fertility. The main goal is crop diversity and the include of legume plants into the production system since they have the ability to improve soil fertility and to stimulate biological activity of soils.

In this study, the effect of two different crop rotation complaxicity on soil health in organic greenhouse vegetable production was evaluated. One consisted of sugar pea as a green manure crop, while the second one with cucumbers and clover intercropped in between crop rows. Samples of soil were collected throughout the growing season and changes in soil fertility were estimated based on analysis of soil nutrients, organic matter content, soil protein, pH, soil respiration and soil microorganism activity.

The results were showed that there were more changes in soil characteristics during the growing season with time than between the two cropping systems. However, both sugar peas and clovers were showed positive impact on soil quality. Both plants improved the level of available nitrogen as well as activity of soil microorganisms, which stressed the importance of legumes for fertile and biologically active soil. Not only did clovers affect soil fertility, but they also served as a ground cover, providing protection of the soil when the crops are growing.

Overall, it can be concluded as the cultivation of legumes is an effective way of maintaining soil quality in organic greenhouses. Legumes supporting the natural process taking place in the soil, and it promote soil fertility.

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Appendix

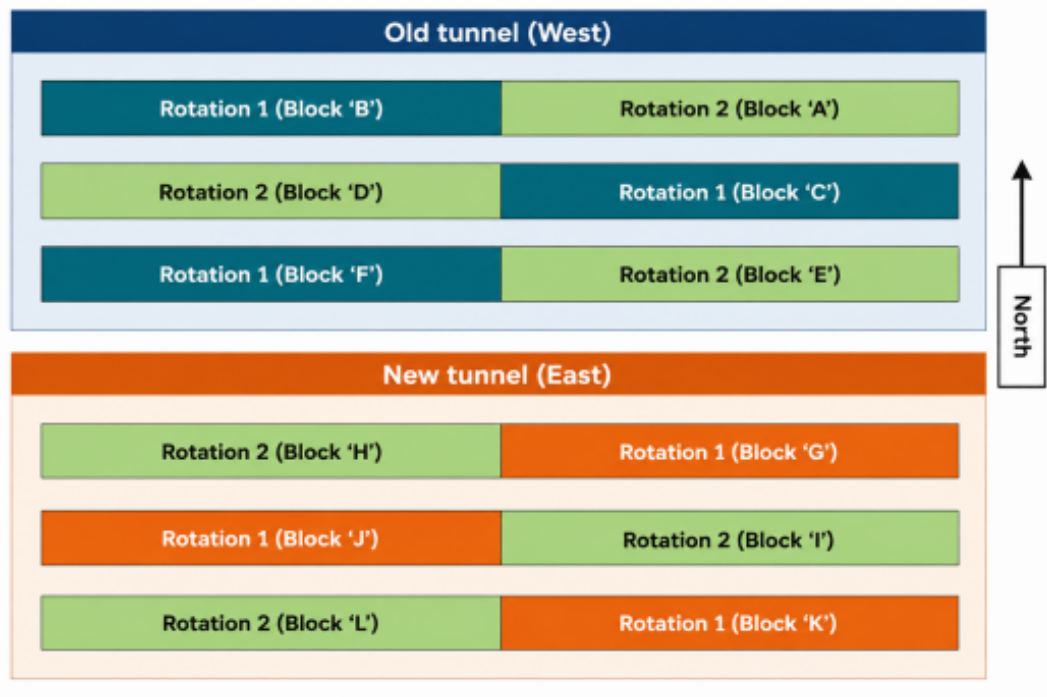


Figure 11 ; Greenhouse Layout and experimental design

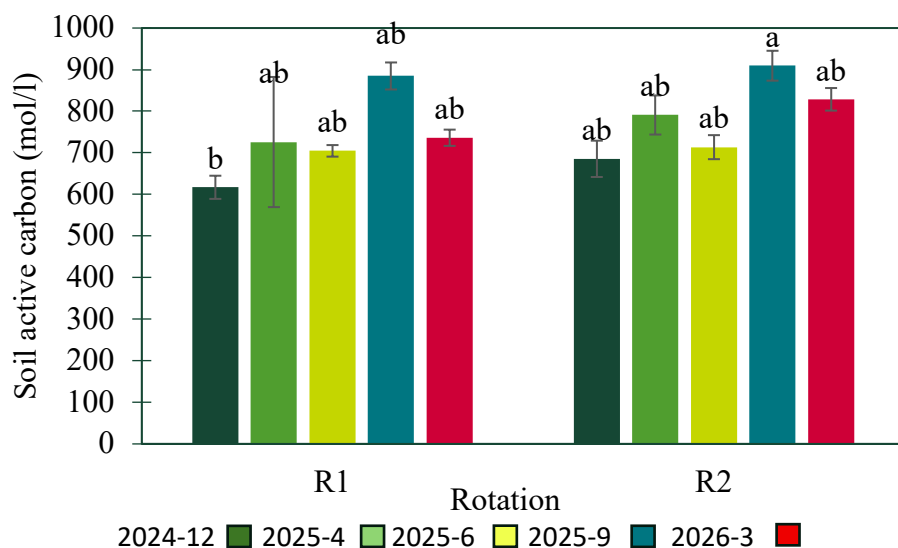


Figure 12 soil carbon content variation between two crop rotations. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models.

Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

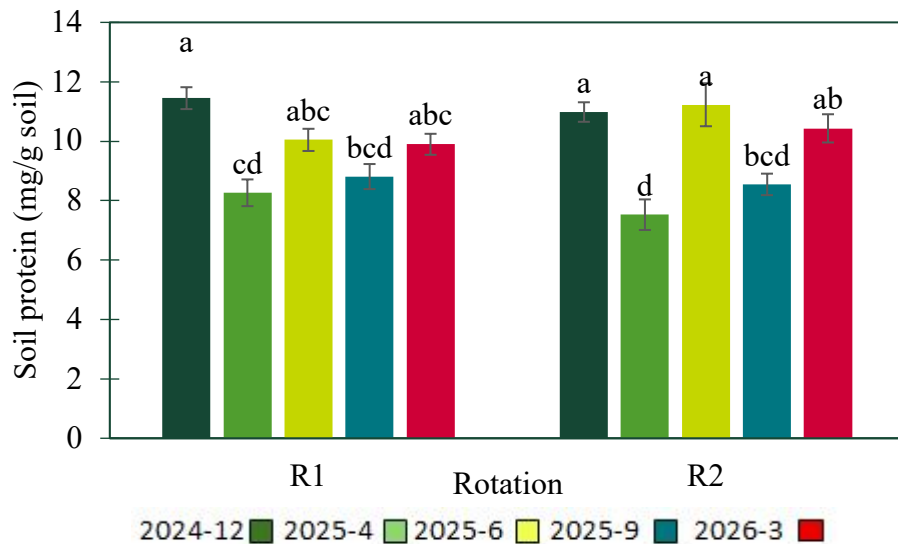


Figure 13; Soil protein content variation between two crop rotations. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$

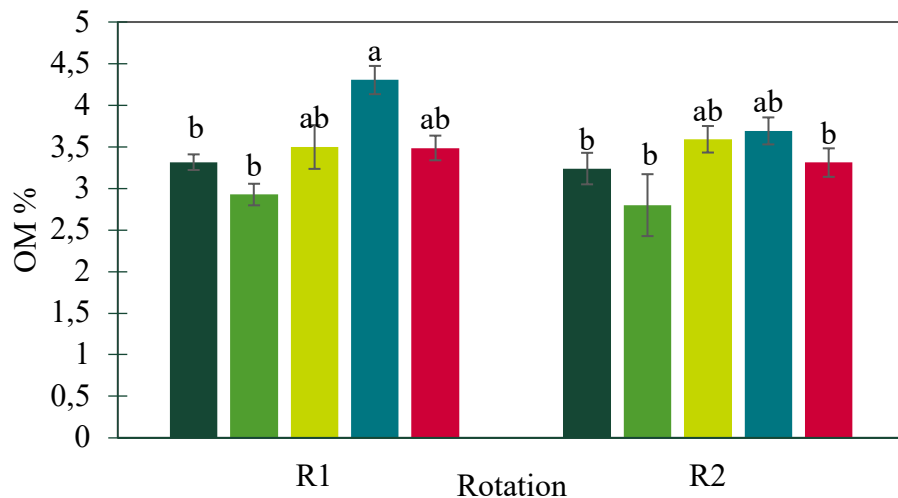


Figure 14; Soil organic matter content variation between two different crop rotations considering the soil sampling dates. Effect of sampling time on Soil protein content. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

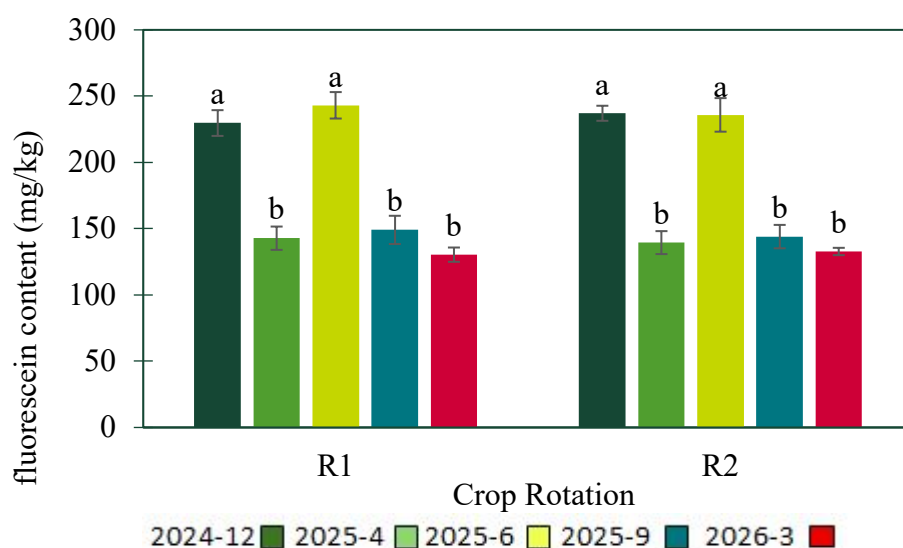


Figure 15; Microbial activity variation between two rotation. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

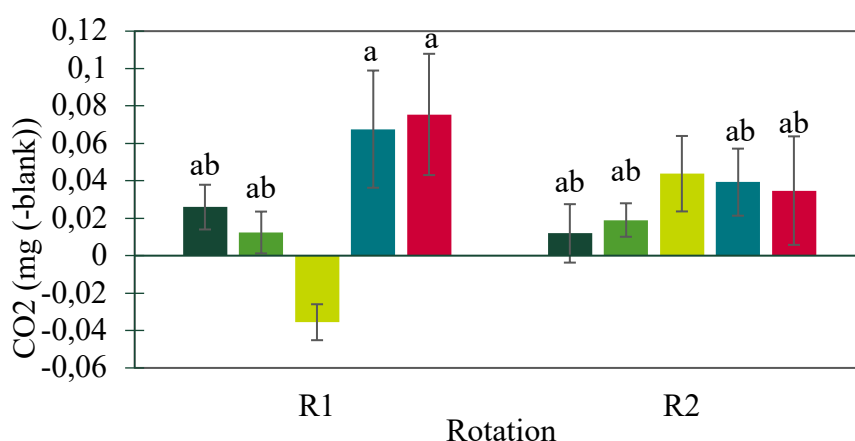


Figure 16; Soil respiration variation between two crop rotations. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

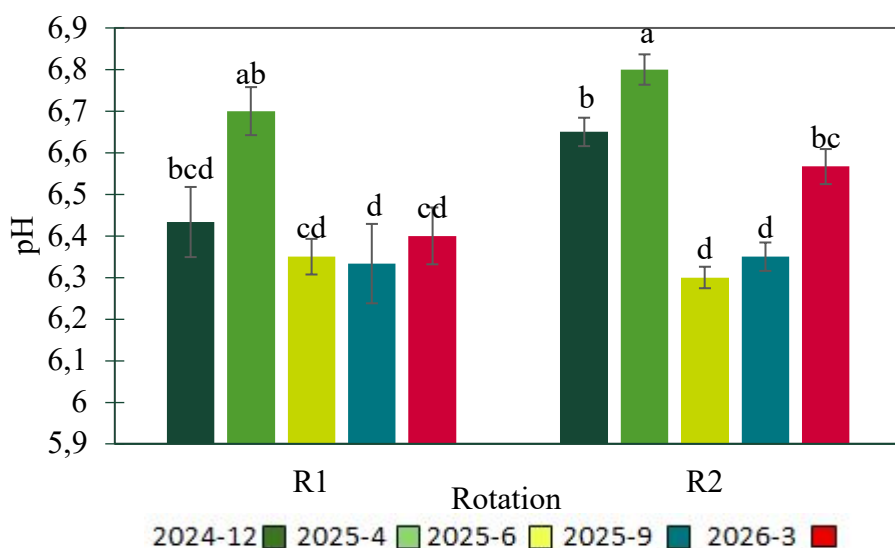


Figure 17; Soil pH variation between two different crop rotations. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

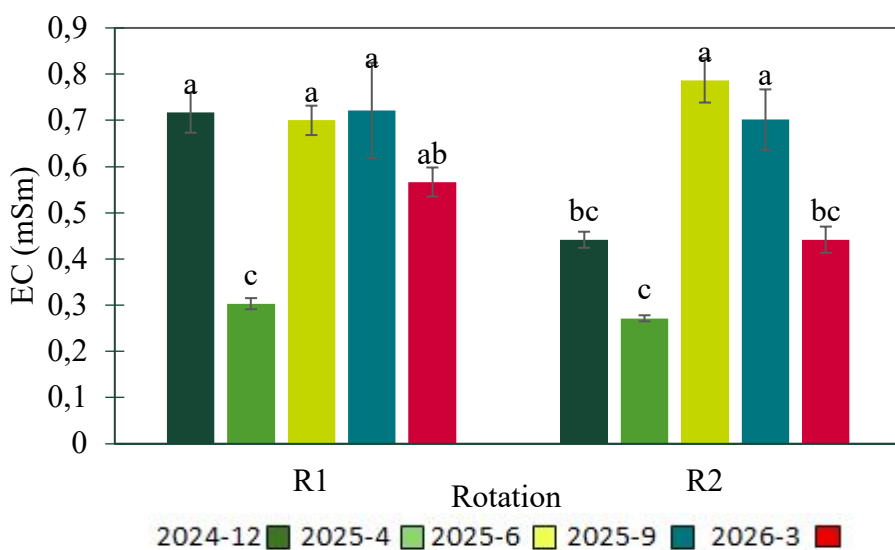


Figure 18; Soil EC variation between two different crop rotations. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

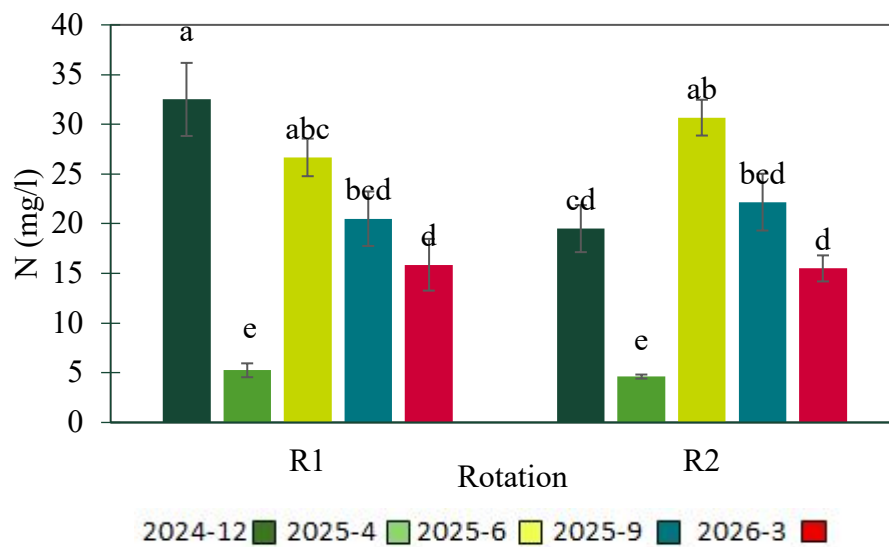


Figure 19; Soil nitrogen content variation between two different crop rotations. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

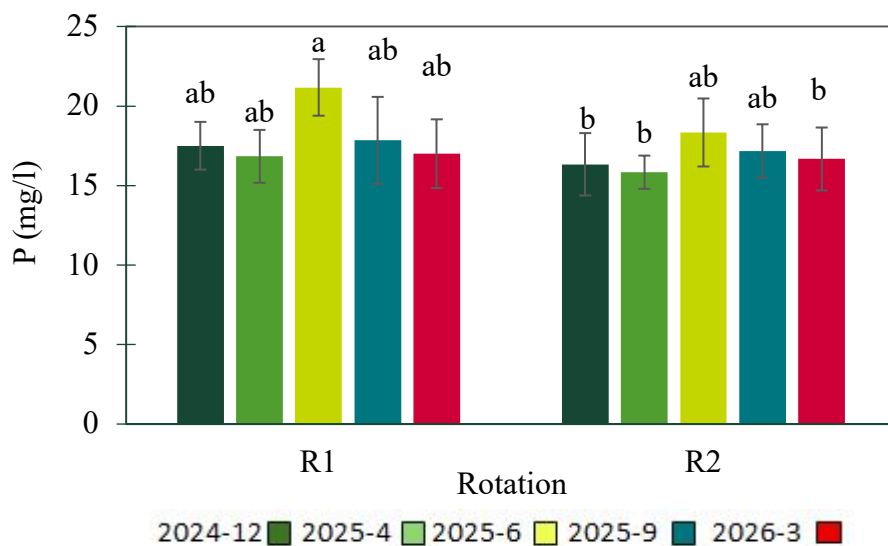


Figure 20; Effect of crop rotation on Soil phosphorus content. Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

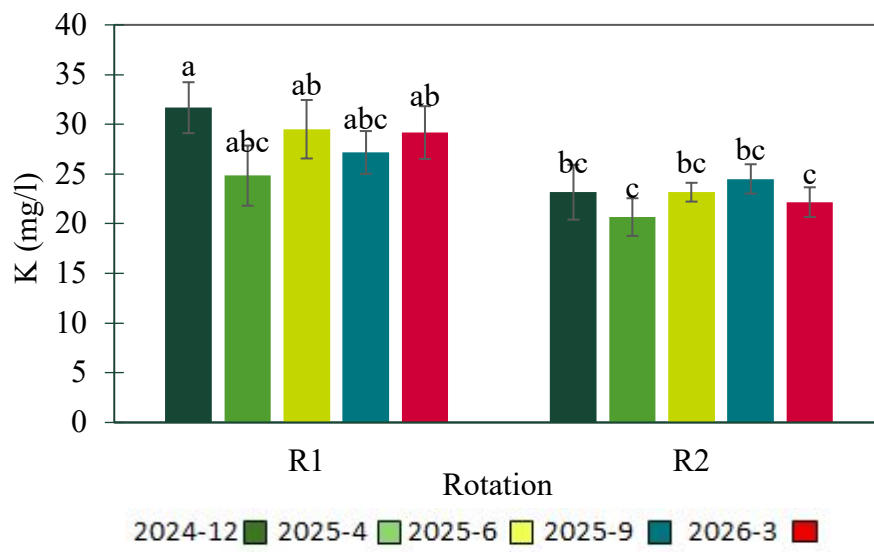


Figure 21; Effect of two different crop rotation on Soil potassium content . Bars represent estimated marginal means $\pm$ standard error obtained from linear mixed-effects models. Means sharing at least one common letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

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