Microbial soil health with legume crop rotation in organic greenhouse production.

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Master Thesis • 30 credits
Swedish University of Agricultural Sciences, SLU
Biosystems and Technology
Horticultural Sciences

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Credits:	30 credits
Level:	Second cycle, A2E
Course title:	Independent project in Horticultural Science
Course code:	EX0948
Programme/education:	Horticultural Science - Master´s Programme
Course coordinating dept:	Department of Plant Breeding
Place of publication:	Alnarp
Year of publication:	2025

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

Swedish University of Agricultural Sciences
Faculty Biosystems and Technology
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Abstract

The purpose of this study is to examine the potential effect the different legume crop rotations have on the microbial soil health in organic greenhouse production. To answer the purpose the following hypothesis was formulated: the microbial soil health is differently affected by the two different legume crop rotations. The method in this study was performed by observing and collecting data from two legume crop rotations: sugar pea (*Pisum sativum* var. saccharatum) as a sole crop, and intercropping cucumber (*Cucumis sativus*) as main crop with crimson clover (*Trifolium incarnatum*) as a green manure crop. Three sets of soil samples were collected to analyse the dynamic and health of the soil before, during and after the growing cycle. The analysis of microbial soil health was based on five indicators: microbial activity, soil respiration, soil protein, available carbon and nutrient analysis. Findings - The results showed a significant difference in sampling dates, but no significant difference was shown between crop rotations. The results also showed that legumes have an effect on the amount of nitrate found in the soil. Hence, this study shows a significant correlation between total amount of nitrate and microbial activity, total amount of nitrate and soil protein, microbial activity (FDA) and respiration. The main finding of this study is that there was not a significant difference in the microbial soil health between the two crop rotations, sole legume crop and intercropped main crop with a legume crop. Thus, the hypothesis for the study was rejected. This study contributes to the knowledge of legumes effect on the microbial soil health in organic greenhouse production, the findings also show the importance of a holistic and societal view taking into consideration factors regarding sustainability and economics

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

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Abbreviations

CASH	Cornell´s Comprehensive Assessment of Soil Health
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FDA	Fluorescein Diacetate Analysis
NPK	Nitrogen, phosphorus and potassium
RQ	Research Question
SLU	Swedish University of Agricultural Sciences

1. Introduction

The soil can be described as the skin of the earth. Hence, a vital part of our ecosystem on which we depend on for our survival. It is therefore of great importance to care for it and understand how to best manage it (Xinghui, et al., 2023; Lehmann, et al., 2020; Moebius-Clune, et al., 2017; Chang, et al., 2022; Doran & Safley, 1997). The FAO predicts that in order to feed the world´s growing population we need to increase our food production with 60% by 2050 (FAO, 2009). In 2023 the USDA published a report on the same subject in which they addressed the increased pressure on our global land to provide food security for a growing population (Sands, et al., 2023). According to Gerten et al. (2020) it is hard to push the world´s feeding capacity with current agricultural practices without significant consequences to the environment and the climate. Global agriculture relies on fertilizers, and they have played a vital part in the last decades increase of global food production capacity (Penuelas, et al., 2023). However, a combination of suboptimal management and extensive use of fertilizers can lead to environmental issues and many parts of the world face problems with these issues such as groundwater pollution, eutrophication, and aquatic dead zones (Xinghui, et al., 2023). Thus, a more sustainable agricultural management is essential to maintain and increase the global food production and food security. Management of soil health is a fundamental part of this approach, since if soil health is not maintained, we risk facing lower yield and degrading quality of our crops lowering productivity levels (Moebius-Clune, et al., 2017). Therefore, it is in both the civil society´s and governing powers interest to find sustainable agricultural practises that can contribute to world-wide food security.

1.1 Soil health

Soil is a complex system and soil health is commonly described as "*the physical, chemical and biological condition of the soil determining its capacity to function as a vital living system and to provide ecosystem services*" (European Commission, 2023; United States Department of Agriculture, 2024). Another definition of soil health is "*the ability of soil to perform or function according to its potential, and changes over time due to human use and management or to natural events*." which highlights the role humans play in soil health (Doran & Safley, 1997). The soil can be divided into different qualities: inherent, dynamic, and factors: physical, chemical and biological (Doran & Safley, 1997; Moebius-Clune, et al., 2017). Inherent qualities referring to factors that are long-term and hard to influence by humans such as soil type (Moebius-Clune, et al., 2017). Dynamic qualities refer to factors that can be directly influenced by human management (Moebius-Clune, et al., 2017). Physical factors are factors such as water capacity, porosity, texture and aggregate size and stability (Moebius-Clune, et al., 2017; Eriksson, et al., 2011). Chemical factors are pH, salinity, anion and cation exchange capacity, and compounds found in the soil such as nutrients (Eriksson, et al., 2011). Biological factors include active carbon, root pathogen pressure and microbial respiration rate (Moebius-Clune, et al., 2017). Soil health is not a new concept and soil health conservation has been known for hundreds of years with records of native American intercropping for an increased soil biodiversity and 18th century scientists discussed soil ecosystems (Ngapo, et al., 2021; Lehmann, et al., 2020). Agricultural sustainability is closely linked with soil health and the maintaining of the soil and the monitoring of key indicators is significant for agricultural productivity (European Commission, 2023). The United States Department of Agriculture describes that there are four principles for soil health management: minimizing disturbance, maximizing living roots, maximizing biodiversity and maximizing soil cover (USDA Natural Resources Conservation Service, 2024). To maintain the ecosystem services that the soil provides it is important to understand that soil management is complex and when improving one factor it can affect other factors in both positive and negative ways (Lehmann, et al., 2020).

1.1.1 Soil health assessment

There are several different indicators that can be used when assessing soil health (Lehmann, et al., 2020; Moebius-Clune, et al., 2017). By using specific methods depending on which factors are interesting for the stakeholder a management plan for improvements can be developed (Moebius-Clune, et al., 2017). Cornell´s Comprehensive Assessment of Soil Health (CASH) and the Soil Management Assessment Framework (SMAF) is currently the two main soil assessment tools (Chang, et al., 2022). The CASH protocol evolved from the SMAF protocol and is of relevance for studies in temperate areas (Chang, et al., 2022). The CASH

protocol holds significance partly for that it takes biological factors into account which has previously been slightly overlooked (Chang, et al., 2022; Lehmann, et al., 2020). It does not however have an assessment for total microbial activity. For total microbial activity is fluorescein diacetate hydrolysis (FDA) a widely accepted method (Adam & Ducan, 2001; Green, et al., 2006; Frlolov, et al., 2022). Whereas previous methods for measuring FDA were not optimized for soil samples Green, Stott and Diack developed a method that is optimized specifically for determining FDA hydrolysis in soil samples (Green, et al., 2006).

1.2 Organic farming

Organic farming is an integrated production management system that aims to have low impact on the environment and use sustainable farming methods (European Commission, 2024; Jordbruksverket, 2024). This is done by, inter alia, conserving or enhancing biodiversity, soil fertility and water quality and use energy and natural resources responsibly (United States Department of Agriculture, 2015; Jordbruksverket, 2024; European Commission, 2024). “Organic” is a globally recognised term and the concept is internationally established (IFOAM, 2024). It is highly regulated and one of few agricultural systems that is closely monitored in detail by governing powers and organisations (EU, 2018/848; National Archives and Records Administration, 2025). The European Union has regulations and policies for producers in the EU and in January 2022 a new legislation was taken in to force for organic farming (EU, 2018/848). For organic farming in greenhouse production the new legislation entails changes. The new regulations state that all organic productions must be produced directly in the ground with contact to the subsoil B horizon and have a crop rotation (Jordburksverket, 2021). Production in greenhouses can no longer be done in containers or demarcated beds except for plants sold in pots (EU, 2018/848). Additionally, the crop rotation must include one type of legume (EU, 2018/848). The organic farming practise has gained recognition as a sustainable form of agriculture and governing powers are aiming for an increased use of the system (Borghino, et al., 2024; IFOAM, 2020). The European Union has a target that 25% of the Union´s agricultural lands should be organically farmed, and the Swedish government’s aim is that 60% of all public meals should be organically produced by 2030 (IFOAM, 2020; Regeringskansliet Näringsdepartementet, 2019). Organic farming imposes challenges such as lower food production and securing enough nitrogen supply while simultaneously matching crop uptake with supply to not risk nutrient loss in the growing system (Borghino, et al., 2024; Röös, et al., 2018). The regulations, international recognition, challenges and governing powers push for increased use makes organic farming an interesting system to study in order to deepen the knowledge of soil health management in organic systems.

1.3 Legumes

Nitrogen is a vital part of the plant, and it can be found in many chemical compounds in the plant cells (Barker & Bryson, 2006). Fertilizing with nitrogen leads to an increase of productivity in most systems, which attest to the importance and need of nitrogen as a plant nutrient (Barker & Bryson, 2006; Taiz, et al., 2018; Xinghui, et al., 2023). It is a well-known fact that plant growth and development as well as productivity are affected when essential nutrients are insufficiently supplied or available (Xinghui, et al., 2023). Even though there is a large amount of nitrogen in the biosphere there is often a need for a nitrogen fixation process in order to break the covalent bond between the nitrogen atoms and make the compound available for the plant (Taiz, et al., 2018). Some bacteria have the ability to fix nitrogen from the atmosphere. This characteristic has led to that a symbiosis can be found between some plants and bacteria where the plant receives available nitrogen and the bacteria other nutrients and carbohydrates from the plant through root exudates (Taiz, et al., 2018). This symbiotic relationship between plant and bacteria is commonly found in the *Fabaceae* family, where the plants form root nodules which the nitrogen- fixing bacteria colonize (Taiz, et al., 2018; Hasanuzzaman, et al., 2020). An incorporation of plants belonging to the *Fabaceace* family into the crop rotations has shown a positive impact on the biological soil health and decreasing the need for chemicals (Taiz, et al., 2018; Lötjönen & Ollikainen, 2018). The use of legumes in the crop rotation is one of the main sources for nitrogen in organic systems (Röös, et al., 2018). Nitrogen is released from a *Fabaceae* crop when it starts to decompose and around two thirds of the fixed nitrogen become available in the soil for the next crop (United States Department of Agriculture, 1998). The amount of accumulation and release of nitrogen from the legume crop depends on multiple factors such as the biomass production and the climate (Mirsky, et al., 2016). A higher quantity of biomass generally yields a higher amount of nitrogen, and a later termination of the crop cycle is a contributing factor for maximizing the crop biomass (Mirsky, et al., 2016). The climate effects the nitrogen mobilisation because of the effect it has on legume growth and on the microbes' breakdown of plant tissue (Mirsky, et al., 2016). The decomposition of plant tissue is typically faster in warm and wet conditions while slower in cold and dry (Mirsky, et al., 2016). Several studies (Diacono, et al., 2021; Ditzler, et al., 2021) have previously shown positive effect of crop rotations with legumes and intercropping legumes with a main crop, however studies comparing the soil health of legumes as a sole crop with intercropped legumes in organic greenhouse production are scarce.

1.4 Purpose and research questions

The aim of this study is to explore the microbial soil health of two different crop rotations including legume crops. The purpose is to examine the potential affect the different legume crop rotations have on the microbial soil health in organic greenhouse production. This is done by observing two different crop rotations. One with sugar pea, as a legume in the crop rotation, and one with intercropping of cucumber, as main crop, and crimson clover, as green manure crop and legume. To help answer the purpose the following research question (RQ) was posed:

RQ: Do the two legume crop rotations affect the microbial soil health in greenhouse production differently depending on the cropping system: intercropping or grown as a sole crop?

1.5 Hypothesis

H: The microbial soil health is differently affected by the two different legume crop rotations.

1.5.1 Delimitations

This study is delimited to an experiment during one growing season with two crop rotations in an organic greenhouse production in the south of Sweden using the two legumes sugar pea and crimson clover comparing the result. The test site on which the experiment is conducted have previously been used for different rotation experiments and may have an impact on the result of this study.

2. Methodology

2.1 Research design

2.1.1 Experimental set-up

This study is an experimental study examining the potential impact legumes have on soil health in organic greenhouse production. It also compares the microbial soil health of two growing systems: crop rotation with sugar pea (*Pisum sativum* var. saccharatum) (V1) and intercropping with cucumber (*Cucumis sativus*) as main crop intercropped with crimson clover (*Trifolium incarnatum*) as a green manure legume crop (V2). The sugar peas and the cucumbers were sown and pre-cultivated in a greenhouse before planted in the tunnel greenhouse. The clover was directly sown in the tunnel greenhouse. The analysis of microbial soil health was based on five indicators: microbial activity, soil respiration, soil protein, available carbon and nutrient analysis. The laboratory work for this project was conducted in SLUs Biosafety level (BSL) 2 laboratory at the department of Biosystems and Technology in Alnarp, Sweden. The project was conducted at Mellangård, Lomma, Sweden in a polytunnel built on a field that has been used for organic production for the past 10 years. The site has previously also been used for different rotation experiments. The greenhouse production area was divided in 6 rows with 2 block per row (block A-L) creating 6 replicates of each rotation. In each row there was one block of sugar pea succeeded by cucumber (rotation 1, V1) and one block of cucumber and clover intercropped growing (rotation 2, V2) (see appendix 1 for greenhouse schematic). All rows are equipped with irrigation.

2.1.2 Plant management

The crops were planted, cared for and monitored in the greenhouse by Alnarp´s Agroecology Farm. The crops were watered with the irrigations system upon demand. Alnarp´s Agroecology Farm administered fertilizer to the crops on four separate occasions during the growing season (table 1). The time of the fertilization was in correlation with the different growing phases of the cucumber crop, vegetative phase and maturing phase. This means that the intercropped rotation V2 which had cucumber for the entire experiment received fertilized three time while crop rotation V1 received fertilizer once after the crop change from sugar pea to cucumber. The amount of fertilizer applied was done in accordance with the

suppliers' recommendation.

Table 1: The date, type of fertilizer and crop rotation that was fertilized.

Date	Rotation	Fertilizer	NPK
June 4, 2024	V2	ECOR 3	12-0-3
June 24, 2024	V2	ECOR 3	12-0-3
July 14, 2024	V2	VIVIKALI	2-0-20
July 19, 2024	V1	ECOR3	12-0-3

2.1.3 Soil sampling

To analyse the dynamic and health of the soil before and after the growing cycle of the crops three sets of soil samples were taken: April 24, 2024, June 20, 2024, September 02, 2024. The sampling times were selected based on the crop rotation. The first sample was taken on April 24, 2024, in the pre-cultivated phase in the beginning of the project before planting the crops. The second sample was taken on June 20, 2024, after the first crop cycle for crop rotation V1 was complete, and the sugar peas were replaced with cucumber. The third and final sample was taken after all the crop cycles for both rotations were completed on September 02, 2024.

2.2 Analyses

2.2.1 Microbial activity (Fluorescein Diacetate Analysis)

The microbial activity was measured using fluorescein diacetate activity analysis (FDA) optimized for soil samples (Green, et al., 2006). One g of air-dried soil was put in a 100 ml reagent bottle. Fifty ml of 60 mM sodium phosphate buffer (Na_3PO_4*$12H_2O$, pH 7.6) and 0.5 ml 4.9 mM FDA lipase substrate solution ($C_{24}H_{16}O_7$) was added to the glass bottle. The mixture was swirled around and then incubated for 3 h at 37 °C. After the incubation 2 ml of reagent grade acetone was added to the solution to stop the FDA hydrolysis. Approximately 30 ml of the solution was transferred to a 50 ml centrifuge tubes and centrifuged for 5 min 8820g. The solution was then filtered through a Whatman No. 2 filter paper [CAT No.1001-150, Cytiva, Shanghai, China] and 1 ml was transferred to a cuvette. Using a spectrophotometer [LPV440.99.00001, DR3900, Hach, Düsseldorf, Germany] the solutions absorbance was measured at 490nm. Controls were performed with every analysis. Instead of adding FDA lipase substrate solution 0.5 ml of reagent grade acetone was added to the control sample. The amount of fluorescein released in the

soil samples was calculated by producing and referencing a standard curve for fluorescein (Appendix 2).

2.2.2 Soil respiration

The soil respiration analysis was performed according to the CASH protocol (Moebius-Clune, et al., 2017). When performing this analysis wide-mouthed 500 ml glass jars with screw tops were used as a sealed chamber. Twenty g of dry soil was weighed and then put into aluminium weighing boat with pre-perforated holes. The weighing boats were placed in the glass jars on top of 2 Whatman filter papers [CAT No.1001-150, Cytiva, Shanghai, China], 7 cm in diameter. On top of the soil sample were a contraption, constructed of chicken wire and plastic lid, placed to work as a tripod for a 10 ml beaker. The 10 ml beakers were duck tapped to the plastic lid of the tripod for extra stability. Aliquots of 9 ml of 0.5 M potassium hydroxide (KOH), acting as CO_2 trapping solution, was added to the 10 ml beakers on top of the tripod. To rehydrate the soil 7.5 ml ddH_2O was added to the filter paper by gently dispensing it on the wall of the glass jar. A blank was made the same way as the samples but excluding the soil. This was done to measure the amount of CO_2 that was trapped in the jar at the start of the analysis. After construction, the jars were closed securely with the screw top lids making them airtight and they were incubated for 93 h in a fume hood at room temperature. After the incubation the jars were opened, and the conductivity of the KOH was measured using a conductivity meter [HQ440D.98.00012, HQ440d multi conductivity meter, Hach, Düsseldorf, Germany]. The soil respiration measured in amount of CO_2 was calculated by subtracting the blank samples trapping solutions conductivity value from the soil samples values. The values were then compared with the conductivity of pure 0.5 M KOH and the conductivity of 0.25 M potassium carbonate (K_2CO_3) which simulates a fully saturated trap solution of KOH.

2.2.3 Soil protein

The soil protein analysis was based on the CASH protocol (Moebius-Clune, et al., 2017). Three g of air-dried soil were weighed out and transferred to 100 ml reagent bottles. Aliquots of 24 ml extractant (20 mM sodium citrate, pH 7.01) were added to the bottles using a pipette boy. The solutions were then shaken on a shaker [0025002982, HS 501 D, IKA, Staufen im Breisgau, Germany] for 5 min at 180 rpm. They were then swirled, and the lid of the bottles were loosened to avoid pressure build up. The solutions were then autoclaved and left to cool to room temperature. After cooling the solutions were swirled in the bottle to resuspend the soil from the bottom. Approximately 1.75 ml solution was drawn from the bottles whilst avoiding the lipid surface and the soil at the bottom of the bottles. The solutions were then put in microcentrifuge tubes and centrifuged for 3 min at 10,000g. Aliquots of 0.8 ml of the centrifuged solution was transferred to storage tubes and refrigerated overnight. The next day the samples were taken out of the fridge and

placed on the counter to allow time to equilibrate to room temperature before the analysis continued. Aliquots 10 µl of the soil sample solutions and standards [23209, Albumin Standards, Thermoscientific, Rockford,IL, USA] were transferred into wells in a 96 well microtitre plate and 200 µl of BCA working reagent [23227, Pierce BCA Protein Assay Kit, Thermoscientific, Rockford, IL, USA] was pipetted in every well. The reaction plate was sealed and put in a water bath for 60 min at 61.5 °C. After the incubation the plate was cooled for 10 min and read in a spectrophotometer [A51119700, Mulitskan SkyHigh, Thermoscientific, Singapore] at 562 nm. Using the standards a regression line was created and the amount of protein in the soil was calculated using the given formula in the standard operation protocol (Moebius-Clune, et al., 2017) (Appendix 3).

2.2.4 Active Carbon

The active carbon analysis is based on the CASH protocol (Moebius-Clune, et al., 2017). A 0.02 M $KMnO_4$ solution was made by mixing $KMnO_4$ with $CaCl_2$ and distilled H_2O and adjusting it to pH 7.2. Due to the solution's light sensitivity the bottle with the solution and the falcon tubes used were covered in aluminium foil. Two and a half g of air- dried soil samples were measured and put in the opaque falcon tubes. In a sequence 18 ml of distilled water was added thereafter. In the same sequence the redox reaction was started by adding 2 ml of the 0.02 M $KMnO_4$ solution to the tubes. The tubes were then shaken on a shaker [0025002982, HS 501 D, IKA, Staufen im Breisgau, Germany] for 2 min at 120 rpm. After the 2 min the tubes were removed from the shaker without stopping the clock, and put on the bench-top for settling for 8 min. After a total reaction time of 10 min 500 µl of the samples were transferred into a new flacon tube containing 49.5 ml of distilled water which stopped the reaction. All the tubes were then shaken by hand for 10 s. After transferring 1 ml to the cuvette the samples were measured in a spectrometer [LPV440.99.00001, DR3900, Hach, Düsseldorf, Germany] at 550nm. To interpret the data a standard curve was created by making a standard dilution series of $KMnO_4$ and measuring it at 550 nm in the spectrometer (Appendix 4). To convert the data into active carbon content the formulas in the CASH protocol were followed (Moebius-Clune, et al., 2017).

2.2.5 Nutrient analysis

A standard analysis of plant available nutrient was performed using Spurway analysis. This was done out-of-house by sending 500 g of each soil sample to the company LMI AB located in Helsingborg, Sweden. The nutrient analysis measured pH, conductivity and the amount of total nitrate, ammonium, phosphorus, potassium, magnesium, sulphur, calcium, manganese, boron, iron, sodium and aluminium (Appendix 5).

2.2.6 Statistical analysis

To assess the difference and determine statistical significance of the data collected from the different soil analyses analysis of variance (general linear model, ANOVA) was performed in Minitab version 19.2020.1.0. Rotation and date were factors and the result from the soil analyses the response. To compare specific group differences the Tukey honestly significant difference test (Tukey´s HSD) was used. To determine any correlation between the different soil analyses a Pearson correlation test ($\alpha = 0.05$) was conducted. All the statistical figures presented in the study were created in Minitab and Excel.

3. Empirical results

3.1 Microbial activity (Fluorescein Diacetate Analysis, FDA)

The amount of fluorescein found in the soil samples ranged from 90.69 to 299.80 mg/kg. (figure 1). The highest FDA values were obtained at the second date of soil sample collecting June 20, 2024, for both rotations and the lowest at the first soil sample date April 24, 2024. At the last and third date of soil sample collecting September 02, 2024, there was a decline of FDA compared to the second date June 20, 2024. This resulted in a significant difference between the soil collecting dates ($p<0.001$). The ANOVA analysis showed that there was no significant difference between the crop rotations when all sampling occasions was combined ($p=0.221$). However, when the soil sample date September 02, 2024, was run by itself it showed significant variance between the rotations ($p=0.049$). Similar results, no significant difference for crop rotation with all the samplings occasions but significant difference for the rotations for the third soil sample September 02, 2024, were found in the Tukey test for rotation. The Tukey test showed a significant difference between the three different sampling dates.

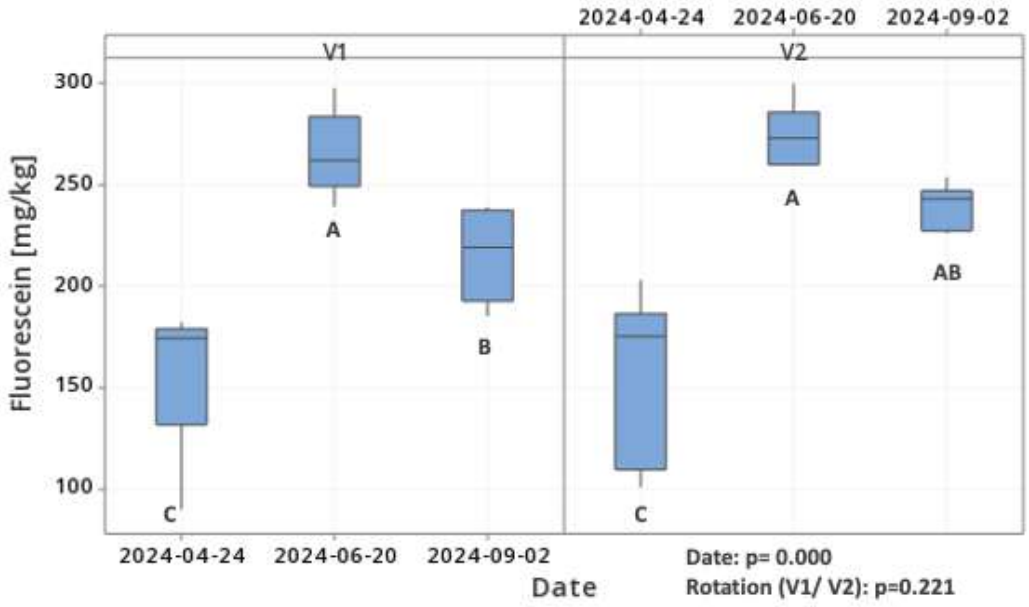

*Figure 1: The amount of Fluorescein (mg/kg) in the two different crop rotations (V1/ V2 at three sampling dates (April 24, June 20, September 02). GLM ANOVA P-value for date and rotation and Tukey comparison grouping for date*rotation. Groupings in the Tukey test that do not share the same letter are significantly different (α = 0.05). Crop rotation V1 is sugar pea succeeded by cucumber and crop rotation V2 is cucumber intercropped with clover.*

3.2 Soil respiration

The soil respiration measured in microbial release of CO_2 values ranged from 0.004 to 0.234 mg/kg soil between the different rotations (figure 2). The respiration values declined from the first soil sampling date April 24, 2024, with the lowest values found in the second soil sample date June 20, 2024. The ANOVA test showed a significant difference for the soil sample dates ($p<0.001$). The two different rotations (V1: sugar pea succeeded by cucumber and V2: cucumber intercropped with clover) did not show any significant difference ($p=0.994$). The Tukey test supported this result. Furthermore, the Tukey comparison showed that the first soil sample date April 24, 2024, were significantly different to the two later soil sample dates June 20, 2024, and September 02, 2024.

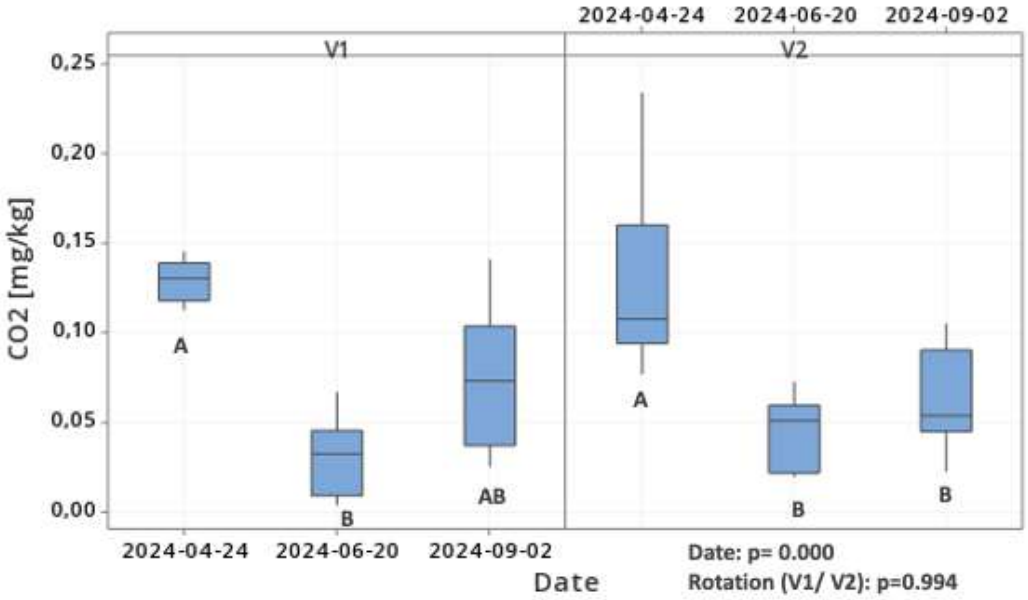

*Figure 2: Amount of CO_2 (mg/kg) in the two different crop rotations (V1/ V2 at three sampling dates (April 24, June 20, September 02). GLM ANOVA P-value for date and rotation and Tukey comparison grouping for date*rotation. Groupings in the Tukey test that do not share the same letter are significantly different (α = 0.05). Crop rotation V1 is sugar pea succeeded by cucumber and crop rotation V2 is cucumber intercropped with clover.*

3.3 Soil protein

The highest amount of soil protein was observed in the second soil sample date 2024.06.20 while the lowest values were measured in the third soil sample date 2024.09.02. The amount of soil protein found in the soil spanned from 5.72 to 11.39 mg/g. Comparing the different sampling dates a significant difference was observed (p=0.005). No significant difference was observed comparing the crop rotations (V1: sugar pea succeeded by cucumber and V2: cucumber intercropped with clover) (p=0.320). The Tukey test observed no difference in rotation either but showed a significant difference between soil sample dates. The third date of the soil sampling 2024.09.02 differed from the first April 24, 2024, and the second June 20, 2024, sample date.

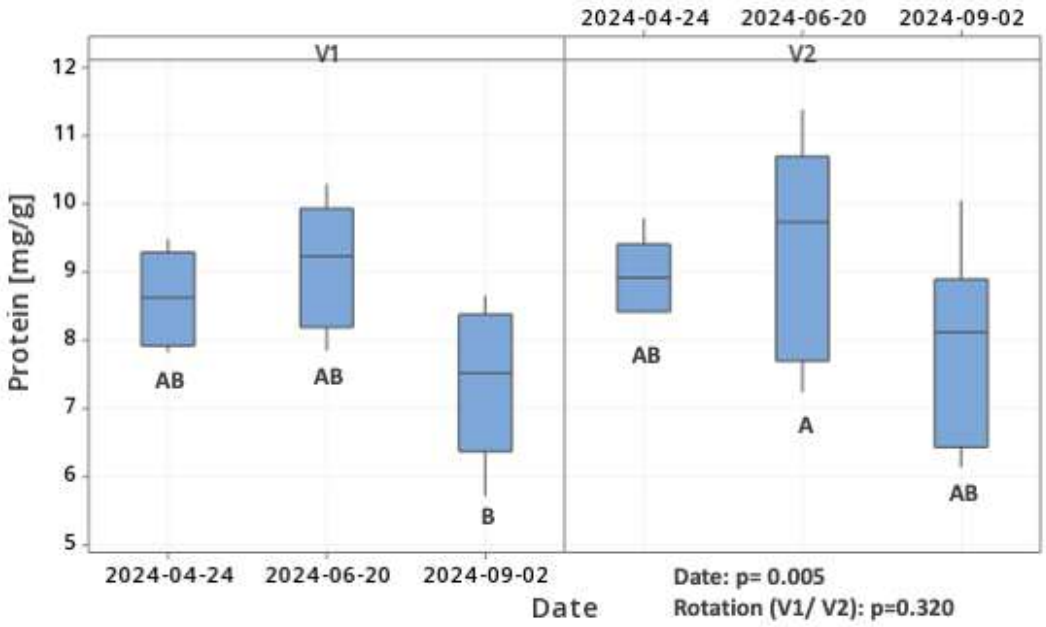

*Figure 3: Amount of protein (mg/g) in the two different crop rotations (V1/ V2 at three sampling dates (April 24, June 20, September 02). GLM ANOVA P-value for date and rotation and Tukey comparison grouping for date*rotation. Groupings in the Tukey test that do not share the same letter are significantly different (α = 0.05). Crop rotation V1 is sugar pea succeeded by cucumber and crop rotation V2 is cucumber intercropped with clover.*

3.4 Active Carbon

The active Carbon values had an increase from the first soil sample date to the third with values spanning from 522 to 788 mg/kg (figure 4). No significant difference was observed in either date (p=0.060) nor crop rotation (V1: sugar pea succeeded by cucumber and V2: cucumber intercropped with clover) (p=0.346) in the ANOVA test. Notably, even though the ANOVA showed no significant results showed the Tukey test significant difference between the first sampling date, April 24, 2024, and last sampling date, September 02, 2024.

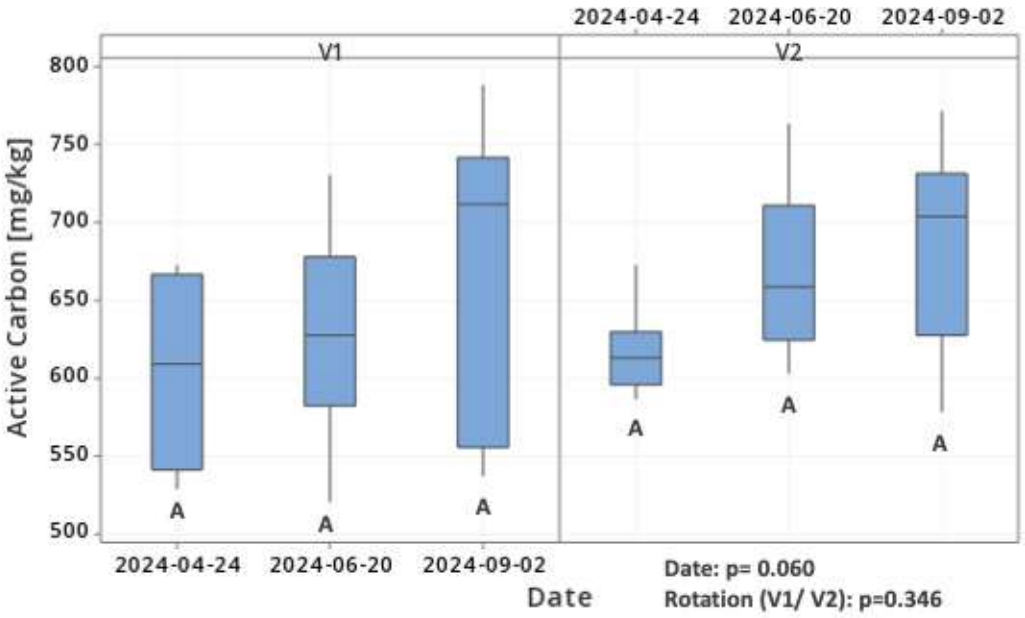

*Figure 4: Amount of active Carbon (mg/kg) in the two different crop rotations (V1/ V2 at three sampling dates (April 24, June 20, September 02). GLM ANOVA P-value for date and rotation and Tukey comparison grouping for date*rotation. Groupings in the Tukey test that do not share the same letter are significantly different (α = 0.05). Crop rotation V1 is sugar pea succeeded by cucumber and crop rotation V2 is cucumber intercropped with clover.*

3.5 Nutrient analysis

Following the methodology described in the statistical analysis section the nutrient raw data was analysed. For complete listing of the results for all the nutrients see Appendix 5. The next four sections will describe in depth the results for nitrogen (nitrate), phosphorus, potassium (NPK) and pH.

3.5.1 Total Nitrate

The highest values of total amount of nitrate were observed at the second soil sampling date June 20, 2024, and lowest measured in the first soil sampling date April 24, 2024 (figure 5). The amount of nitrate found in the soil ranged from 11 mg/l to 57 mg/l. When an ANOVA was performed the results showed that there was significant difference between sampling dates ($p<0.001$) while there was no significant difference between crop rotations (V1: sugar pea succeeded by cucumber and V2: cucumber intercropped with clover) ($p=0.051$). The post hoc Tukey test showed no difference in rotation but for soil sampling dates. The second soil sampling date June 20, 2024, was significantly different from the two other soil sampling dates April 24, 2024, and September 02, 2024, which were placed in the same grouping.

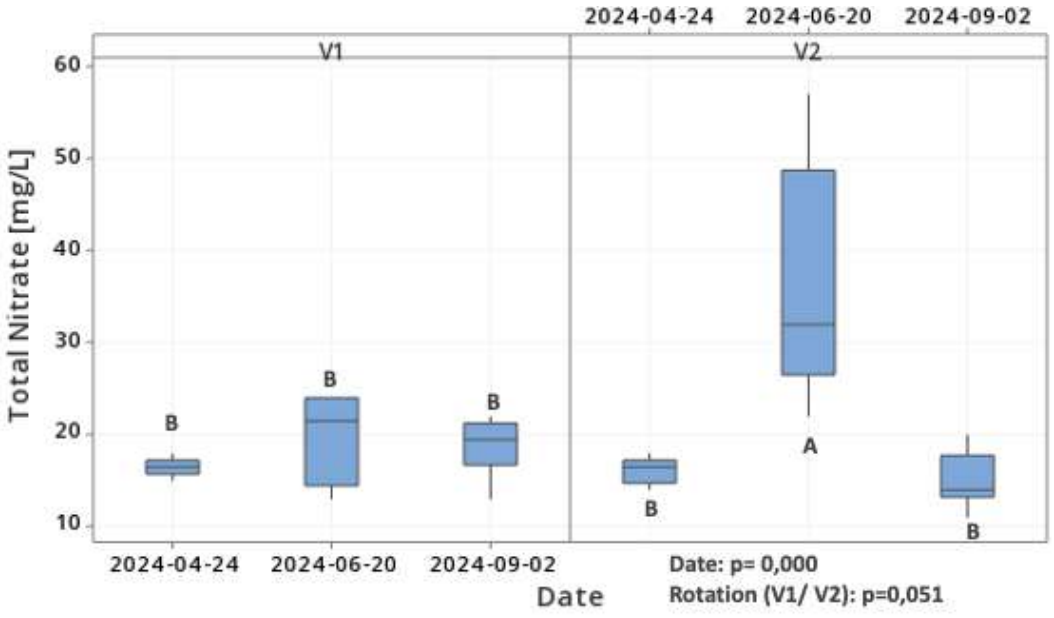

*Figure 5: Amount of total Nitrate (mg/l) in the two different crop rotations (V1/ V2 at three sampling dates (April 24, June 20, September 02). GLM ANOVA P-value for date and rotation and Tukey comparison grouping for date*rotation. Groupings in the Tukey test that do not share the same letter are significantly different (α = 0.05). Crop rotation V1 is sugar pea succeeded by cucumber and crop rotation V2 is cucumber intercropped with clover.*

3.5.2 Phosphorus

The second soil sampling date, June 20, 2024, had the highest overall value while the first sampling date, April 24, 2024, the lowest (figure 6). The overall values for phosphorus ranged from 10 mg/l to 24 mg/l. No significant variances were observed for neither soil sampling date (p=178) nor rotation (V1: sugar pea succeeded by cucumber and V2: cucumber intercropped with clover) (p=0.965) in the ANOVA test. The Tukey test further supported the ANOVA result with no significantly different grouping of the date and rotation compared with the amount of phosphorus.

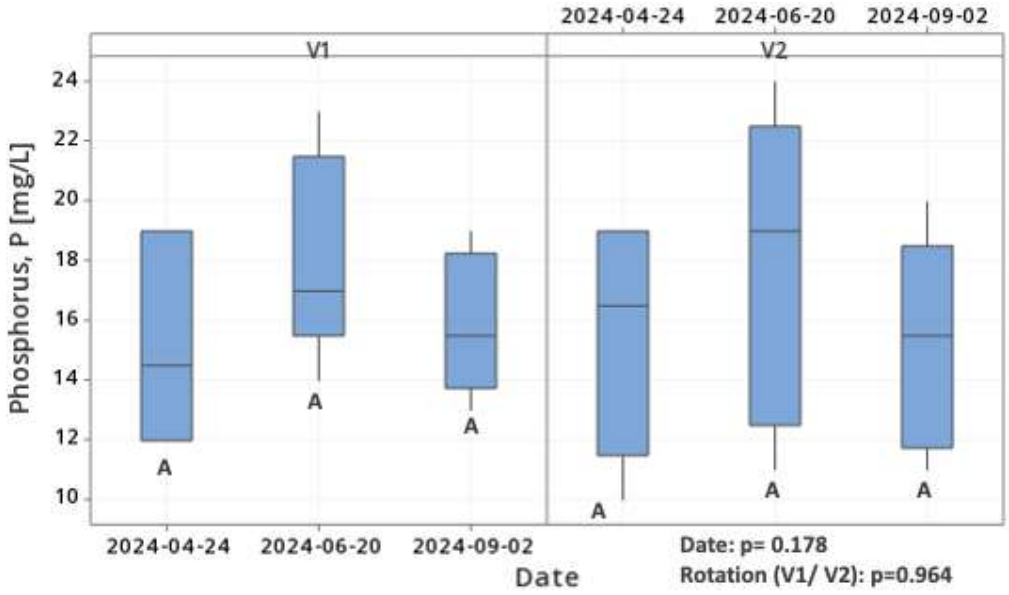

*Figure 6: Amount of Phosphorus (mg/l) in the two different crop rotations (V1/ V2 at three sampling dates (April 24, June 20, September 02). GLM ANOVA P-value for date and rotation and Tukey comparison grouping for date*rotation. Groupings in the Tukey test that do not share the same letter are significantly different (α = 0.05). Crop rotation V1 is sugar pea succeeded by cucumber and crop rotation V2 is cucumber intercropped with clover.*

3.5.3 Potassium

The rotations (V1: sugar pea succeeded by cucumber and V2: cucumber intercropped with clover) had the opposite amounts of potassium with a decline in values for V1 from the first sample date April 24, 2024, to the third September 02, 2024, and an increase of values for V2 from the first sample date April 24, 2024, to the third September 02, 2024. The measured overall value for potassium spanned from 18 mg/l to 40 mg/l. The amount of potassium showed no significant difference for the soil sample dates (p=0.420) or the crop rotation (p=0.077). This was result was reflected in the Tukey test with no significant groupings observed.

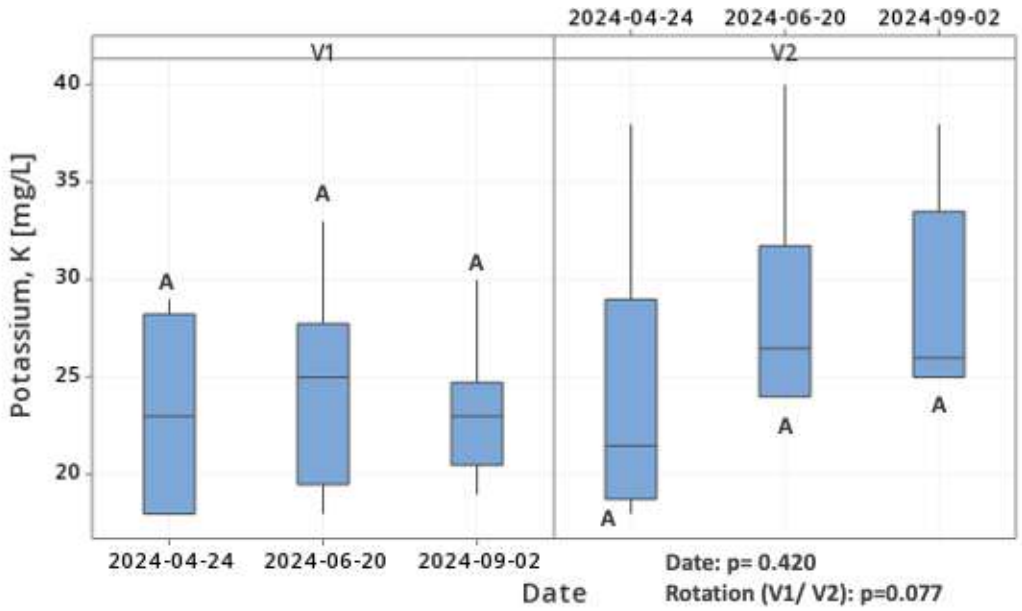

*Figure 7: Amount of potassium (mg/l) in the two different crop rotations (V1/ V2 at three sampling dates (April 24, June 20, September 02). GLM ANOVA P-value for date and rotation and Tukey comparison grouping for date*rotation. Groupings in the Tukey test that do not share the same letter are significantly different (α = 0.05). Crop rotation V1 is sugar pea succeeded by cucumber and crop rotation V2 is cucumber intercropped with clover.*

3.5.4 pH

The pH was overall the highest in the first sampling date April 24, 2024, and the lowest in the second sampling date June 20, 2024. The measured pH value spanned from 5.9-6.6. The ANOVA test followed by Tukey did not show any significant difference for the different sampling dates (p=0.211). The rotation did however show significant difference (p=0.027). The same results were reflected in the post hoc test.

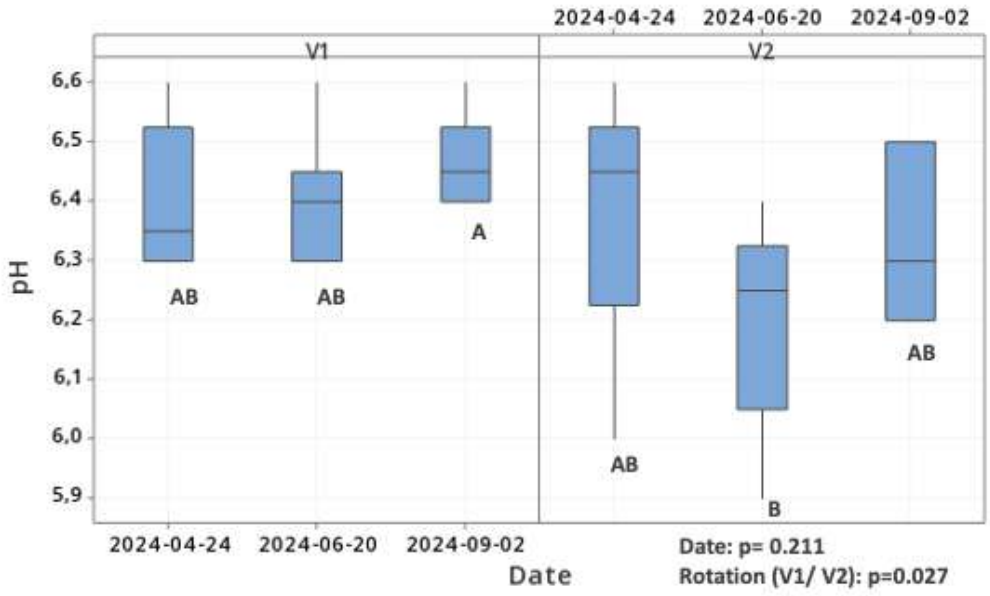

*Figure 8: pH level in the two different crop rotations (V1/ V2 at three sampling dates (April 24, June 20, September 02). GLM ANOVA P-value for date and rotation and Tukey comparison grouping for date*rotation. Groupings in the Tukey test that do not share the same letter are significantly different (α = 0.05). Crop rotation V1 is sugar pea succeeded by cucumber and crop rotation V2 is cucumber intercropped with clover.*

3.6 Correlation

The correlation between all the analyses presented in the previous sections of the result chapter are presented in a correlation matrix (figure 8) showing mixed result. The FDA had a positive correlation with all the other factors except for with respiration (CO_2) which had a negative correlation. This was the lowest value of the entire test (r =-0.780). The FDA and the total of nitrogen showed the highest positive coefficient correlation (r=0.436). The soil protein showed a positive to the other factors. Soil respiration (CO_2) and pH had an overall negative correlation with the other factors except for between each other. The significance of the correlation is showed by the correlation p-values (figure 9). The test showed a significance for the pairwise comparing of FDA and respiration (CO_2) ($p<0.001$), FDA and total nitrate ($p=0.008$); soil protein and total nitrate ($p=0.011$), respiration (CO_2) and total nitrate ($p=0.041$), respiration (CO_2) and phosphorus ($p=0.020$), phosphorus and total nitrate ($p=0.040$), pH and soil protein ($p=0.001$) and pH and total nitrate ($p=0.001$).

Table 2: Pearson correlation test and p-values showing pairwise correlation of fluorescein (mg/kg), soil protein (mg/g), CO2, active carbon (mg/kg), total nitrate (mg/l), phosphorus (mg/l), potassium (mg/l and pH). Red shades indicate positive correlations, blue shades indicate negative correlations, and darker colours reflect stronger relationships. Lighter or neutral colours suggest weaker or no correlation.

		FDA [mg/kg]	Protein [mg/g]	CO2 [mg/kg]	Active Carbon [mg/kg]	Total Nitrate, N [mg/L]	Phosphorus, P [mg/L]	Potassium, K [mg/L]	pH
FDA [mg/kg]	r	1,000							
	p-value	*							
Protein [mg/g]	r	0,099	1,000						
	p-value	0,566	*						
CO2 [mg/kg]	r	-0,780	-0,063	1,000					
	p-value	0,000	0,717	*					
Active Carbon [mg/kg]	r	0,254	0,327	-0,284	1,000				
	p-value	0,135	0,051	0,093	*				
Total Nitrate, N [mg/L]	r	0,436	0,419	-0,343	0,194	1,000			
	p-value	0,008	0,011	0,041	0,257	*			
Phosphorus, P [mg/L]	r	0,173	0,245	-0,386	0,201	0,343	1,000		
	p-value	0,314	0,150	0,020	0,241	0,040	*		
Potassium, K [mg/L]	r	0,209	-0,008	-0,009	0,163	0,243	-0,228	1,000	
	p-value	0,220	0,964	0,957	0,342	0,153	0,182	*	
pH	r	-0,147	-0,539	0,199	-0,146	-0,548	-0,171	-0,100	1,000
	p-value	0,392	0,001	0,245	0,394	0,001	0,318	0,562	*

Pearson Correlation (r)

-1	-0,5	0	0,5	1

4. Discussion

In this study the potential effect different legume crop rotations have on the microbial soil health was examined. To ensure food production and food security the management of soil health is crucial. This study shows that there were no significant differences in the crop rotations across the soil analyses except for the pH test. Thus, the hypothesis, stating that there would be a difference between the different crop rotations, was not supported. Hence, the results show that the different legume crop rotations did not affect the microbial soil health in organic greenhouse production differently. However, it could be argued that the results still give a relevant contribution for this research field.

The results in this study are in line with previous studies confirming that legumes have an effect on the amount of nitrogen found in the soil (Taiz, et al., 2018; Hasanuzzaman, et al., 2020; Mirsky, et al., 2016). Moreover, the result shows an increase of total nitrate at the second soil sampling when the sugar peas were mulched down and replanted with cucumber (figure 5). However, the cucumber and clover crop rotation showed high values for the second soil sampling and did not have a change in crop or any mulching. The correlation between the mulching and the amount of total nitrate is therefore hard to prove and might be due to the fact of the application of fertilizer in the cucumber and clover rotation (table 1) and not the use of legumes in the crop rotation. The amount of total nitrate measured followed the same trend as microbial activity, soil protein and phosphorus with the highest values found in the second soil sampling. The lowest values were observed in third soil samples. Additionally, the microbial activity and the total nitrate showed a significant correlation in the Pearson correlation test. Showing that, the amount of organic matter greatly influences microbial activity since, in most environments, it's the primary source of carbon (Maier & Gentry, 2015). The decomposition of organic matter in the soil is mostly done by soil microorganism and the process of this affects the amount of nitrogen available in the soil (Son, et al., 2003). Furthermore, when microbes utilize organic compounds such as soil organic matter, they require phosphorus and nitrogen (Maier & Gentry, 2015). Hence, the pattern of microbial activity, total nitrate phosphorus observed in this study is therefore, based on the fundamentals of heterotrophic microorganisms, entirely plausible. The findings are in line with the CASH protocol stating that the amount of nitrogen in the soil is affected by the microbial activity (Moebius-Clune, et al., 2017).

Furthermore, in addition to following the same trend as total amount of nitrate, phosphorus and microbial activity, the results showed a significant positive correlation between total amount of nitrate and soil protein. The soil protein, which is found in the soils organic matter, is the largest reservoir of microbial available organic nitrogen and is linked with the soils ability to store nitrogen (Moebius-Clune, et al., 2017; Hurisso & Culman, 2021). Moreover, more than 90 percent of the soils total amount of nitrogen is organic nitrogen (Kelley & Stevenson, 1995). This supports the trend and significant correlation between nitrate and soil protein found in this study. The findings in this study are strengthened by previous studies. As Naasko et al. (2023) showed similar results with a positive correlation between total amount of nitrate and soil protein, which they concluded was due to the strong ties between the total amount of soil nitrogen and the organically bound nitrogen Other studies also found a positive correlation between nitrate and soil protein (Geisseler, et al., 2019; Jha, et al., 2022; Liptzin, et al., 2023). It is however notable that soil protein and nitrogen are interlinked, and it is therefore questionable if one can make a correlation between them. This because it raises questions such as if one is an effect of the other, or if they have a common root cause.

Soil respiration gives an indication of the metabolic activity levels of the microorganisms in the soil while the FDA analysis is an indicator of total microbial activity (Green, et al., 2006). The respiration values showed reversed results compared to total nitrate, microbial activity, phosphorus and soil protein with the lowest values found in the second soil sampling. The microbial community is in turn responsible for, among other things, making nutrients available for plants (Moebius-Clune, et al., 2017). It is therefore not unpredicted that the results of the FDA and soil respirations showed a significant correlation p-value ($p<0.001$). However, the result showing a negative correlation ($r= -0.780$) was not expected. Son et al. (2006) also found a correlation between the FDA and soil respiration when they looked at converting agricultural land to natural vegetation (Son, et al., 2006). An interesting finding is that Son et al. (2006) found a positive correlation while this study found negative correlation between the analyses. Son et al. (2006) did however use a different method when measuring the soil respiration and this might factor in with the different findings.

As mentioned in the beginning of the discussion, the pH showed a significant difference between the crop rotations however the measured values were in the optimal range for pH in accordance's to CASH soil health framework (Moebius-Clune, et al., 2017). This indicates that the pH levels are not a big contributing factor in limiting the availability of nutrients for the crops between the different crop rotations. The trend of significant difference between the collecting dates was reoccurring for the other soil analyses, however not for the pH, active carbon, potassium and phosphorus. Showing that even though there is a difference between

crop rotations for the pH analysis the sampling dates did not factor in for the significant difference in crop rotation. Additionally, the result highlights that even though the test show statistical significance it is not always of biological relevance.

It could be expected, in accordance with the hypothesis, that the results in this study should have shown stronger significant differences. One possible explanation to why the results did not indicate this, could be the quality of the crops studied. As the sugar peas had poor growth, and quality and can therefore not be expected to have had a big effect on the microbial soil health. The poor growth of the sugar pea might be due to plant management, weed pressure and climate factors. It is known that stress in plants can affect the performance of legumes and thereby effect the results of this study (Xinghui, et al., 2023). Another influencing factor is that the cucumber plants also received different amount of fertilizer since they were planted at different times. Due to this, the second soil sampling might be hard to draw hard conclusions from. In the results outliers were identified in the raw data. The outliers are not consistent to the same sampling blocks. Therefore, no conclusion can be drawn about the outliers as a cause of previous experiments done on the study site. The outliers could, however, be due to natural distribution, measurement errors and/or unforeseen experimental conditions but to determine this statistically more soil data points are needed. Consequently, the outliers were kept in the study. Furthermore, factors such as climate variations and irrigation management may have impacted the growing system, and as such the results of the study (Rosberg & Alsanius, 2022). Finally, the present study did not monitor agronomic data such as crop development, yield, plant biomass, climate data and irrigation, this data could contribute in order to make a full-scale conclusion of the legumes impact on the microbial soil health.

To reflect on the social, ethical and sustainable aspect. Microbial soil health is crucial for sustainable crop production, supporting nutrient cycling and ecosystem resilience. Optimizing soil health management promotes long-term soil fertility and contributes to environmental sustainability. Socially, sustainable agricultural practices empower farmers and promotes local communities by increasing knowledge, local food security and job security. Ethically, perusing better agricultural practises shows a commitment to environmental stewardship and intergenerational responsibility. In order for the research and development to be authentic and credible; transparency, validity and responsibility is of the outmost importance.

4.1 Future research

This study could be argued to deepen the knowledge in the field and shows the need for further studies. For future research, it could be suggested to further examine if the result in this study is due to the different crop rotations or the specific crops used in V1/V2. Arguing how it could be beneficial to use the same legume crops in both crop rotations. When using the same legume crops in both rotations you isolate the variability allowing further comparability between the different crop systems since different legumes varieties could fix different amounts of nitrogen. Thus, allowing further analysis and the potential optimization of crop variation and/or crop combination, contributing to further increase of soil health. In future research, one factor to take into consideration for the choice of legume crop, is the economic aspect. Implementation of legumes in the crop rotation must not lead to economic loss for the growers as it then could lead to decline of the production and counteract the goals of organic farming with consequences such as fewer organic farmers on the market. Thus, the legume crop needs to either be intercropped with their cash crop or be a cash crop by itself. This highlights the importance of further research of optimal crop choice for a more sustainable market. Furthermore, while this study shows that legumes influence the soil health it also shows that time span of the crop cycle is a significant variable. Since commercial greenhouse crop cycles are generally short, it is uncertain if the crop cycles timespan in the crop rotation. is optimal to obtain the soil health benefits of legumes. It could be argued that this is a factor which the EU legislation (2018,848), that states the need for legumes in the crop rotation in organic greenhouse production, fail to take into consideration. Therefore, the basis for the legislation of legumes in organic greenhouse production could argued to be uncertain. Future studies are needed focusing on finding the optimal time span of the crop cycle needed to obtain maximal soil health benefits from the legume crops. In pursuance of determining if there is sufficient basis for the legislation in its current state. Finally, future studies are suggested to also consider agronomic data such as crop development, yield, plant biomass, climate data and irrigation for a more full-scale analysis.

5. Conclusion

Based on the findings in this study following conclusions are drawn. Firstly, this study shows that there was no significant difference in the microbial soil health between the two different crop rotations, sole legume crop and intercropped main crop with a legume crop. Secondly, the study showcases that there is a significant difference for most of the soil analyses for the date of the soil samples. Showing that the time span of the crop cycle is a significant variable in organic greenhouse production. Thirdly, this study shows that it is evident to understand the interactions of the factors in the growing system. While this study contributes to the knowledge of legumes effect on the microbial soil health in organic greenhouse production, the findings also show the importance of a holistic and societal view taking into consideration factors regarding sustainability and economics. A holistic view is also important when discussing legislations concerning crop production since crop production is a complex system, thus continuous development supported by science is essential.

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Popular science summary

The soil is a vital part of our ecosystem on which we depend for our survival. It provides the foundation for food production and sustains a vast array of organisms. Degradation of soil health through unsustainable agricultural practices not only threatens biodiversity but also compromises the resilience of ecosystems in the face of climate change related to sustainable development. To feed the growing population global food production must increase with 60 % by 2050 according to FAO, highlighting the great importance of soil health. Since the sustainable management of soil health is needed to ensure food production and food security. This can however not be sustainably done with conventional agriculture which heavily rely on chemicals for fertilizing among other things. The use of chemical fertilizer in agriculture has led to consequence such as ground water pollution and eutrophication. Incorporating legumes in the crop rotation has shown a positive impact on the microbial soil health and lowering the need for chemical fertilizer. This study explores the microbial soil health of two different crop rotations with legume crops. This was done in an organic greenhouse setting where soil samples were taken and analysed. The result showed, what previous study also found, legumes have an impact on the soil health. The amount of nitrogen measured in the soil was higher after planting legumes than before. However, it did not show a difference between the two legume crop rotations but showed that time was a significant factor. This study contributes to the knowledge of legumes effect on the microbial soil health in organic greenhouse production and is one step in the right direction for a more sustainable management of soil health. Addressing these challenges is essential for the long-term sustainability of both human societies and the ecosystem.

7. Learning process

Throughout the course of this project, I have gained valuable insights into my own skills, knowledge, and development needs. Conducting independent research showed the importance of deepening my knowledge of plant science, experimental design and agricultural practises. I started this project with a foundational knowledge of soil health, but I realized that I needed to enhance my skills in data analysis, scientific writing, and laboratory work with different instruments.

Writing this thesis highlighted personal growth, particularly in critical thinking, problem solving and work endurance. Collaborating with my advisor taught me communication skills and how to convey complex ideas more clearly.

Overall, this thesis journey has expanded my reflection skills, technical knowledge, and helped me identify the importance of continued skill development for a successful future in horticultural research and practice.

8. Sustainability contribution

This study contributes to the horticultural sector through increased knowledge of legumes effect on the microbial soil health in organic greenhouse production. The soil is a vital part of our ecosystem, and the management of soil health is a cornerstone in sustainable and responsible use of our ecosystems. This in line with Agenda 2030 and sustainable development goal 15/Life on land (United Nations, 2025). Soil health management is also key for an increased food security. This is study builds knowledge around responsible agricultural practises. This is in line with Agenda 2030 and sustainable development goal 13/Climate change (United Nations, 2025).

Acknowledgements

Firstly, I would like to thank my supervisor Anna Karin Rosberg for her support, guidance and knowledge, throughout this project.
To all my family and friends, I would like to express a loving thank you for believing in me and for supporting me through this journey.
My ever-devoted studying partner, Mini the cat, I am grateful for your company and support.
Lastly, I would like to thank my partner, Kristoffer Alm for motivating and pushing me while highlighting my love for science.

Appendix 1

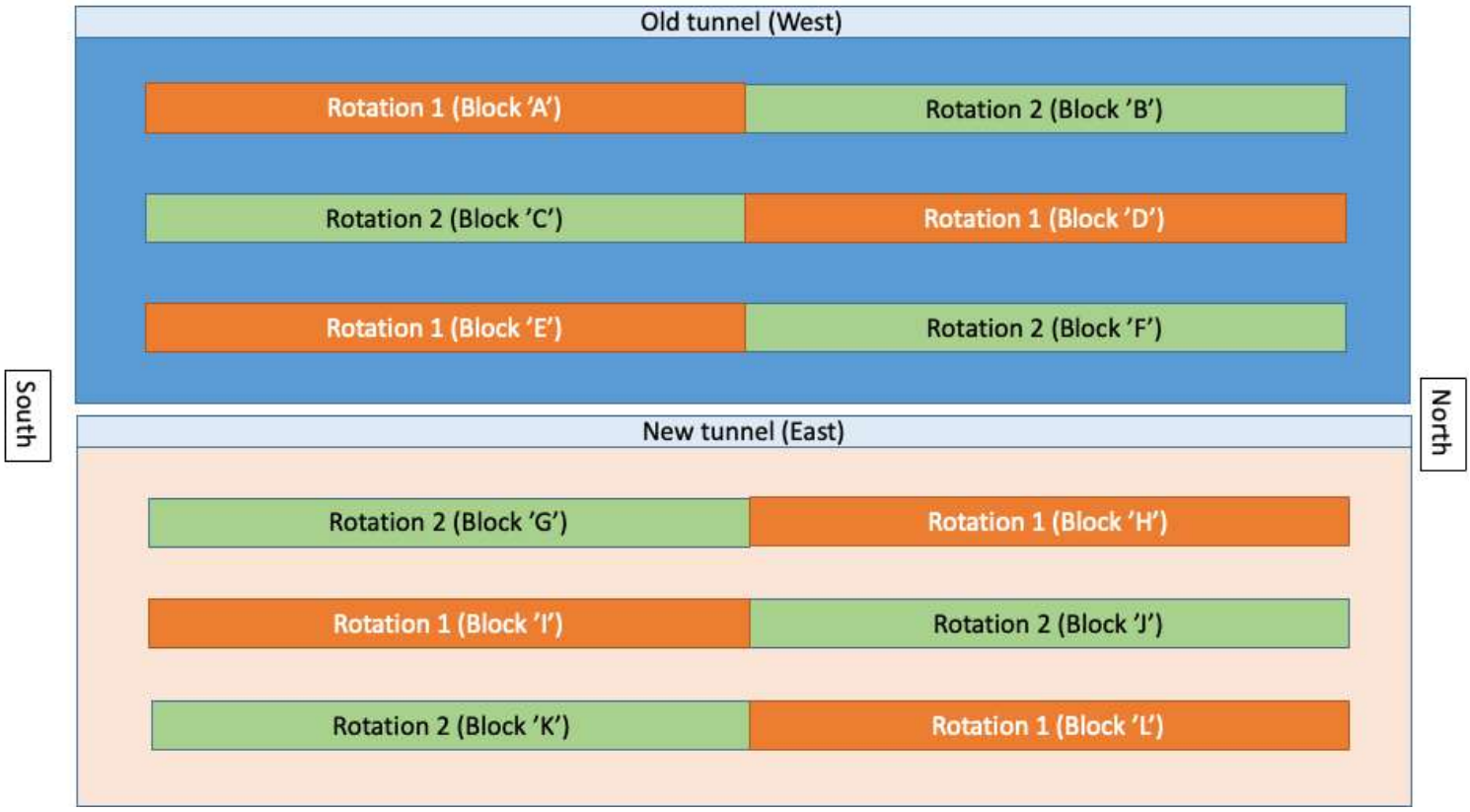


Figure 9: Greenhouse schematic showing the different rotation and blocks.

Appendix 2

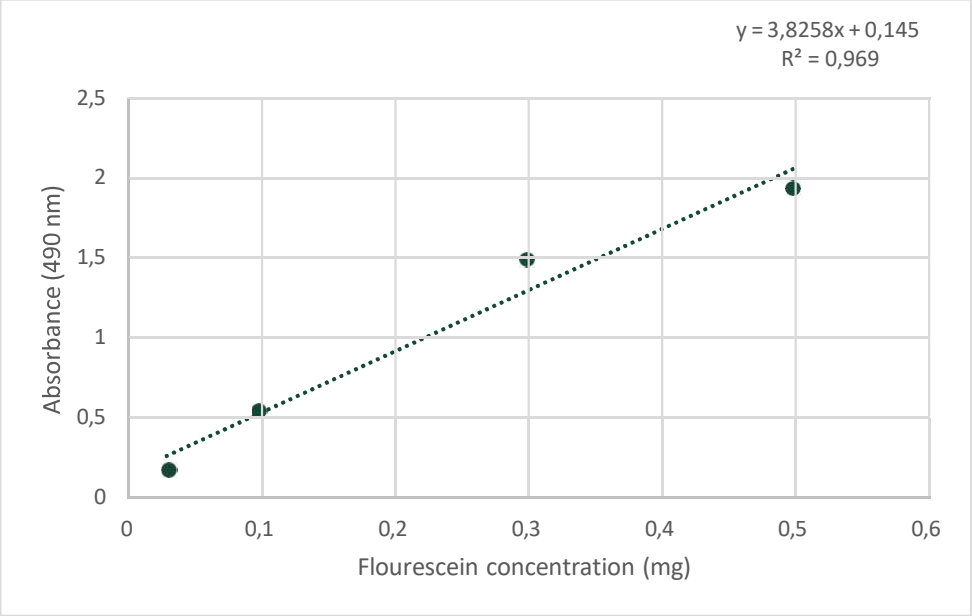


Figure 10: FDA standard curve.

Appendix 3

Soil sample 1	20240424	
a	b	c
-3,27976E-07	0,00226472	0,20556308
5,10819E-08	0,00010348	0,03801565
0,995884264	0,07673487	
Soil sample 2	20240620	
a	b	c
-2,65132E-07	0,00216916	0,20777744
3,83076E-08	7,76E-05	0,0285089
0,997744077	0,05754544	
Soil sample 3	20240902	
a	b	c
-4,68647E-07	0,00260118	0,19266483
2,80156E-08	5,6751E-05	0,02084948
0,998862575	0,04208483	

Table 3:Second order regression line for the soil protein divided by the soil sample dates.

Appendix 4

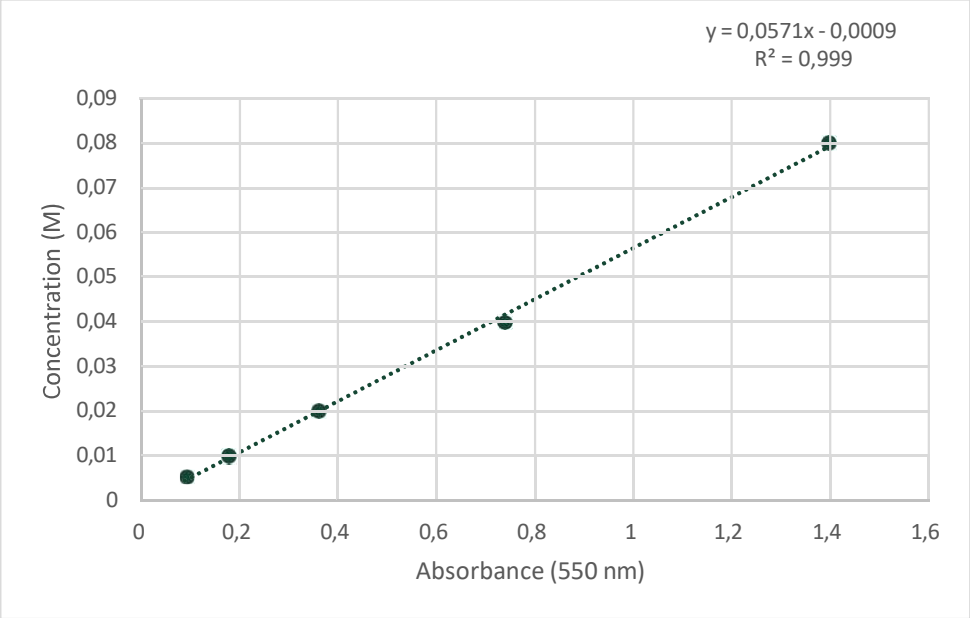
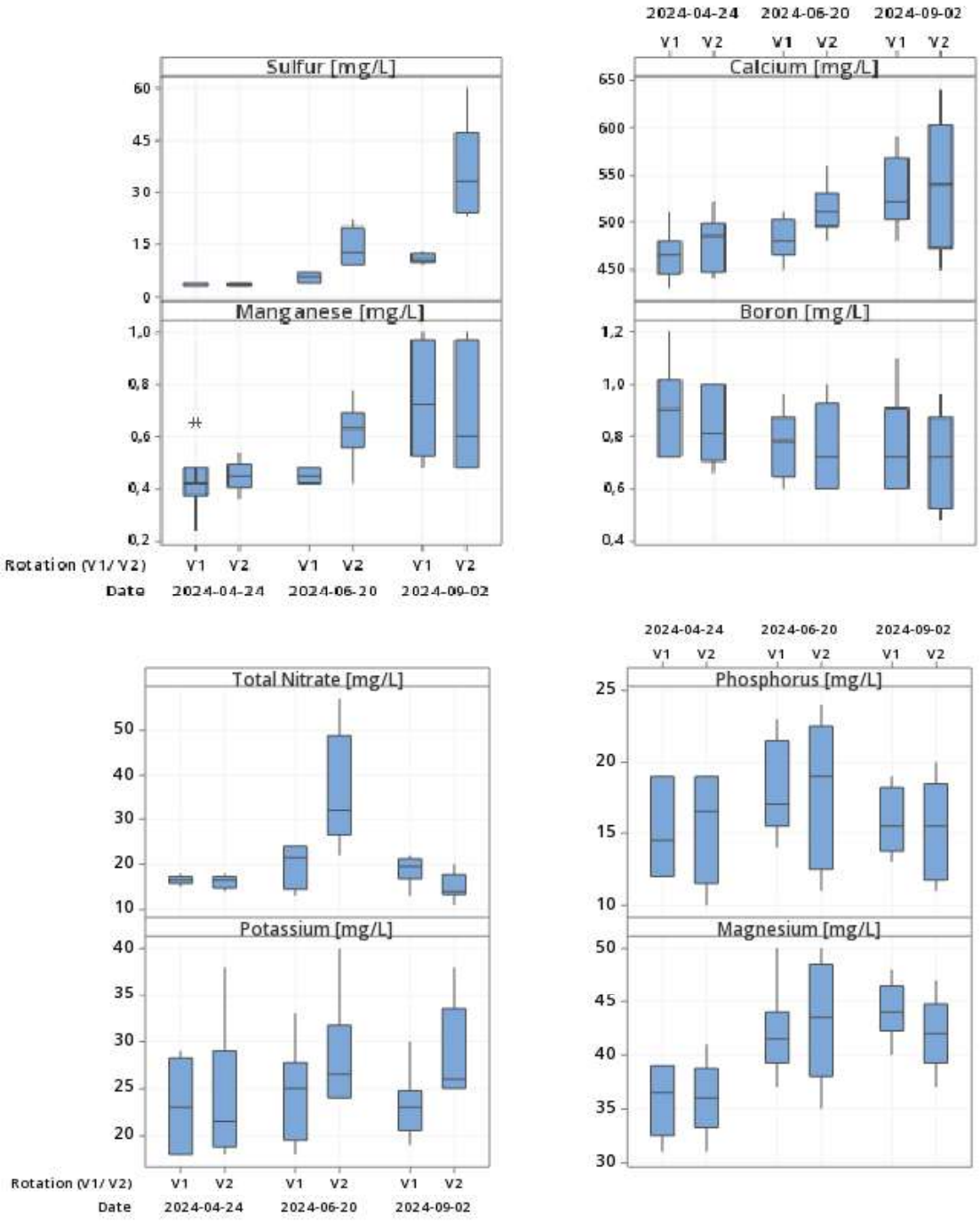


Figure 11: Soil active carbon standard curve.

Appendix 5


Sulfur [mg/L]
Manganese [mg/L]
Calcium [mg/L]
Boron [mg/L]
Total Nitrate [mg/L]
Potassium [mg/L]
Phosphorus [mg/L]
Magnesium [mg/L]
Rotation (V1/V2)
Date
2024-04-24
2024-06-20
2024-09-02
V1
V2

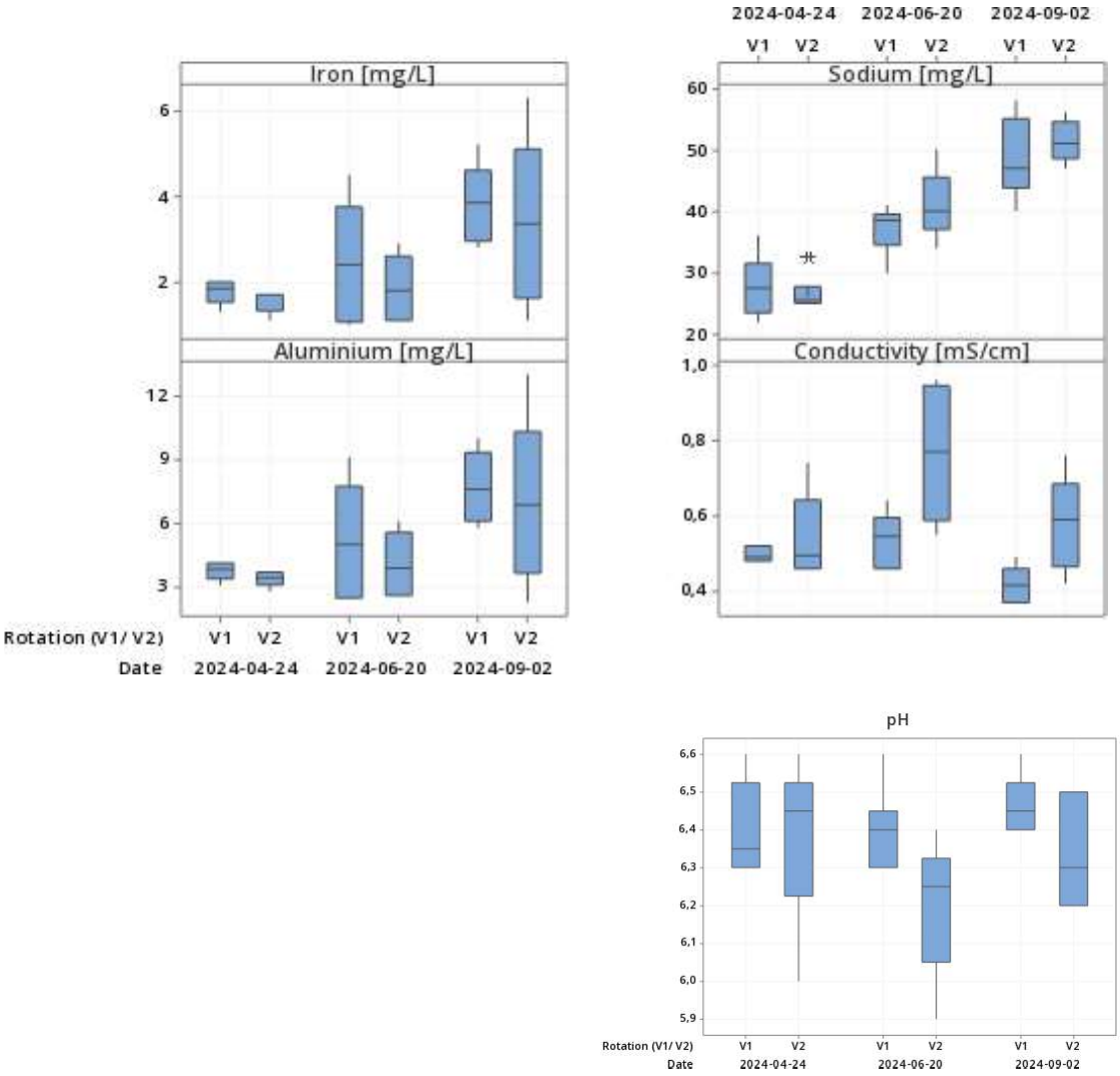


Figure 12: Nutrient analysis values.

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