The impact of the flowering field on soil health and nitrogen dynamics

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Degree project/Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty/Department: Faculty of Landscape Architecture, Horticulture and Crop Production Sciences
Program/Education Horticulture science program
Place of publication Alnarp



The impact of the flowering field on soil health and nitrogen dynamics.

Blommande åkers effekt på markhälsa och kvävedynamiken.

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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
Course coordinating dept:	Department of Plant Breeding
Place of publication:	Epsilon
Year of publication:	2026
Copyright:	All featured images are used with permission from the copyright owner.
Keywords:	Cover crops, nitrogen, nitrate, ammonium, soil health, microbiology, CASH, FDA, POXC, soil respiration, ACE soil protein, flowers, flowering field, dicots, Sweden, Scania

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Abstract

"Flowering field" is a new concept in Sweden in which a farmer receives a subsidy for growing a variety of flowers in the field to support biodiversity. This study examines how farmers can improve the soil from the effects of the flowering fields, offering potential solutions to modern agricultural/horticultural problems, challenges like a changing climate and rising fertilizer costs.

To evaluate soil health, various biological and chemical parameters were analyzed. The experiment compared a flowering field mix *(Red clover, Crimson clover, Persian clover, Yellow clover, Oilseed radish, English ryegrass, common Buckwheat and Lacy Phacelia*) against a traditional cover-crop use (*Spring barley* followed by *oilseed radish*), managed through either ploughing or chemical spraying in the spring.

The result showed that the flowering field had a significantly greater nitrate change after winter; however, this nitrate change was lost later in the season, when the oilseed radish had a higher nitrate level than flower fields, regardless of soil management treatments. Regarding the biological parameters, microbial activity (FDA) showed a significantly greater change in the sprayed oilseed radish treatment than in both the flower mixes. Conversely, active carbon (POXC) had a significantly higher difference in the ploughed flowering field than the sprayed flowering field, but not for the oilseed radish treatments.

This result suggests that while different seed mixes distinctly influence soil health parameters, further research is required to determine the long-term effects of these flower mixes on subsequent crop and soil management. However, a flowering field could be a vital piece of the puzzle in the upcoming agricultural and horticultural system.

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Abbreviations

Abbreviation	Description
ACE	Autoclaved-Citrate Extractable
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of variance
BNF	Biological nitrogen fixation
CASH	Cornell soil health laboratory's comprehensive assessment of soil health standard operating procedures
FDA	Fluorescein Diacetate Analysis
NH_4^+	Ammonium
N^2	Diatomic nitrogen gas
NO	Nitric oxide
N_2O	Nitrous oxide
NO_3^-	Nitrate
PCA	Principal Component Regression
POXC	Permanganate Oxidizable carbon
SMN	Soil mineral nitrogen
SOC	Soil organic carbon

Abstract

"Flowering field" is a new concept in Sweden in which a farmer receives a subsidy for growing a variety of flowers in the field to support biodiversity. This study examines how farmers can improve the soil from the effects of the flowering fields, offering potential solutions to modern agricultural/horticultural problems, challenges like a changing climate and rising fertilizer costs.

To evaluate soil health, various biological and chemical parameters were analyzed. The experiment compared a flowering field mix *(Red clover, Crimson clover, Persian clover, Yellow clover, Oilseed radish, English ryegrass, common Buckwheat and Lacy Phacelia*) against a traditional cover-crop use (*Spring barley* followed by *oilseed radish*), managed through either ploughing or chemical spraying in the spring.

The result showed that the flowering field had a significantly higher nitrate change after winter; however, the amount was lost further into the season, when the oilseed radish had a higher nitrate level, regardless of soil management treatments. Regarding the biological parameters, microbial activity (FDA) had a significantly greater change in the sprayed oilseed radish treatment than in both of the flower mixes. Conversely, active carbon (POXC) had a significantly higher difference in the ploughed flowering field than the sprayed flowering field, but not for the oilseed radish treatments.

This result suggests that while different seed mixes distinctly influence soil health parameters, further research is required to determine the long-term effects of these flower mixes on subsequent crop and soil management. However, a flowering field could be a vital piece of the puzzle in the upcoming agricultural and horticultural system.

1. Introduction

Flowering field and field edges originated as an initiative from farmers in Scania, Österlen (Pågen 2020; Hammarstedt N.D.). As they observed a decline in specific pollinators and wanted to address it, they developed a project to fund the planting of rows of flowers on agricultural land for free. With the aim of increasing biological diversity (Odmark 2020; Natursvårsverket 2024; Jordbruksverket 2026). However, the effects of these fields on soil health and increased production value haven't been fully explained. This work's main objective is to examine the effects of a specific species mix on soil, using nutrient and microbial analyses, and to quantify the value of the flowering field to the sequence crop. Since biodiversity is not only for the overground biomass but also for the belowground, different plants have different functions for gathering different microorganisms (Rodríguez-Gasol et al. 2026). To see if the flowering field can be a piece of the puzzle, solving two big problems in the agricultural and horticultural sectors: the rising prices of fertilizers and a changing climate. Flowering field could possibly be one of many different tools to solve these problems by utilizing different aspects such as nitrogen fixation and soil penetration from different species in the flowering field.

To analyze this, biological, chemical, and physical aspects were measured to get a holistic view of the changes in the soil. The experiment was divided into a flowering mix (*Red clover, Crimson clover, Persian clover, Yellow clover, Oilseed radish, English ryegrass, common buckwheat, and Lacy Phacelia*) and an oilseed radish, and both were planted in a field previously used for spring barley. In the spring, half of the plots were ploughed, and half were sprayed down, and then celeriac was planted in every plot.

1.1 Objective/Aim

The aim of this study is to evaluate the potential benefits of the Flowering field for improving soil health in Scanian conditions. By exploring how farmers can maximize the benefits of this new funding program both environmentally. The study will assess its impact on biological and chemical parameters, such as soil organic matter (SOM), nutrient status, and microbial activity, using analyses to quantify the effects of management and species on soil function.

- Can flowering fields improve the overall soil health for the sequence crop?
- Is the microorganism's activity dependent on N-availability in the soil and the storage for the upcoming crop?
- Can flowering fields increase the N storage in the soil for the upcoming crop, thus reducing the input of synthetic fertilizers?

- Do the ploughed or non-ploughed alter the composition of nitrogen and microbial activity?
- What effect do herbicides have on the nitrogen and microbial activity?

1.2 Hypotheses

Hypothesis 1: Flowering fields exhibited greater change in microbial activity (FDA, ACE Soil protein, Soil Respiration) compared to oilseed radish fields.

Hypothesis 2: Flowering field exhibited greater change of plant available nitrogen after winter and after the treatments.

Hypothesis 3: Ploughed fields exhibit a greater change in microbial activity (FDA, ACE Soil protein, Soil Respiration) after soil cultivation than non-plowed fields.

Hypothesis 4: Ploughed flowering fields exhibited greater change in plant available nitrogen availability than non-plowed fields.

2. Background

2.1 Flowering field “blommande åker” in Sweden

Initial efforts to incorporate flowers into production fields aren't new, but optimizing this approach for specific flower species and their preferences remains underexplored. The flowering field project in Sweden started in 2019 as “Österlen flowering”, initiated by HIR Skåne (Pågen 2020; Hammarstedt N.D). It quickly expanded into “whole Scania flowers” in 2020 and further into “whole Sweden flowers” in 2021 (Svarfvar 2021; Hammarstedt 2024; N.D). The project aimed to increase biodiversity in agricultural landscapes, focusing on pollinators, wild fauna, and natural predators, in response to observed declines (Odmark 2020; Natursvårsverket 2024; Jordbruksverket 2026). In 2025, a new subsidy called 'flowering field and field edges' was introduced to support sowing flowers in arable fields within crop rotations. Jordbruksverket (2025) estimated that about 8,800 hectares would apply for this funding in 2025, but the total applications across Sweden reached 14,594 hectares, a 166% increase over the targeted goal. For 2026, the total area applied to the flowering field is 26,755 ha[1].

2.1.1 Rules and subsidies

The rules are based on different laws, both EU and Swedish. A summary of the rules regarding this subsidy can be found in Appendix 4.

There have been changes in the rules from the 2025 to 2026 season. In 2025, there were no restrictions on how much area you could sow on your land. However, declining cereal prices led some to see it as equally profitable to sow a flowering field rather than spring barley (Nilsson 2025). This appears to be the reason for the change in the regulation for 2026. The rules for the 2026 season were changed from no restriction to a restricted area of 10 hectares plus 20% of the remaining arable land on the farm. The amount farmers can get from this funding depends on where the field is located (Jordbruksverket 2026) Appendix 4; Table 4).

2.2 Crop choice for flowering fields

The regulations set by the Swedish Board of Agriculture (Jordbruksverket) for flowering fields consist of 69 species. The main requirement is to have at least three species that flower during the sowing year (Jordbruksverket 2026). In this study, eight different species were selected to create a multifunctional design and

[1] Lisa Karlsson, Environmental Analysis Unit, Swedish Board of Agriculture, mail, 2026-04-16.

a robust ecological niche strategy to both uphold the subsidy and improve soil health.

The selected crops included Red clover *(Trifolium pratense),* Crimson clover *(T. incarnatum)*, Persian or Reversed clover *(T. resupinatum),* Yellow sweet clover *(Melilotus officinalis),* English ryegrass *(Lolium perenne)*, Oilseed radish or fodder radish *(Raphanus sativus* L. var. *oleiformis Pers)*, Common Buckwheat *(Fagopyrum esculentum)*, and Lacy Phacelia *(Phacelia tanacetifolia).*

The strategy is to utilize the annuals (*Crimson/Persian clover, oilseed radish, Common Buckwheat, and Lacy Phacelia*) to secure the subsidy by ensuring continuous flowering. By offering diverse flower shapes alongside different types of nectar and pollen, they benefit multiple insects. Meanwhile, Red clover, Yellow sweet clover, and English ryegrass are intended to persist growing after the annuals have been mown. These species will then function as a "green bridge" over winter, providing erosion control and nutrient retention. Furthermore, the deep taproots of Yellow sweet clover and Oilseed radish serve to alleviate soil compaction and recycle nutrients from the subsoil. Finally, the inclusion of multiple legumes aims to enhance nitrogen fixation, which is expected to benefit the subsequent crop.

2.2.1 Trifolium spp. (pratensis, incarnatum, resupinatum)

For this flowering mix, three clovers were selected: Red clover *(Trifolium pratense),* Crimson clover *(T. incarnatum)*, and Persian or Reversed clover *(T. resupinatum)*. These clovers serve multiple agroecological purposes, including soil enhancement, green manure, erosion prevention, fodder, pasture, medicinal uses, and providing nectar for pollinators (Fergus & Hollowell 1960; Knight & Hollowell 1974; Peaslee & Taylor 1989; Taylor & Quesenberry 1996; Stout et al. 1997; Lloveras & Iglesias 2001; Khatami et al. 2016; Mardi et al. 2023; Weinert et al. 2023) However, for this study, these species were mainly chosen for their complementary growth habits and their ability to fix atmospheric nitrogen (N^2) through biological nitrogen fixation (BNF). The idea of having three clovers was to diversify different ecological niches and thus increase the flowering field's resilience, allowing it to adapt to varying climatic conditions.

Both Persian and Crimson clover are characterized as long-day annuals (Knight & Hollowell 1958). However, Crimson clover can survive mild Swedish winters, depending on the sowing date (Brandsæter et al. 2002). Red clover, also a long-day plant, is a perennial, meaning it survives winter, and vernalization influences its growth and development (Cumming 1959; Bowley et al. 1984; Ergon et al. 2016). These species support each other phenologically: once the annuals finish flowering and meet the subsidy requirements, they are cut down, while red clover persists. During winter, red clover acts as a "green bridge," reducing exposure of bare soil. Furthermore, even though Persian clover may not

produce the highest seasonal biomass among all clover species, it makes up for it by being one of the fastest-growing clovers with drought resistance (Balazadeh et al. 2021; Ahmadzadeh Chaleshtori & Mohammadi 2025). In contrast, Crimson and Red clover contribute higher biomass later in the season, especially Red clover (den Hollander et al. 2007), creating a resilient system that provides both environmental benefits and climate resistance.

However, differences are not limited aboveground. While all three species develop a taproot with lateral fibrous roots and form symbioses with *Rhizobium leguminosarum* symbiovar *trifolii* (Smith et al. 1982; Moore et al. 2020; Jambagi et al. 2023; Janczarek et al. 2024b; a; Pešić et al. 2025), their root architectures vary. The root systems of the annual species are typically confined to the top 0-20cm of soil (den Hollander et al. 2007; Amsili & Kaye 2021), whereas Red clover can extend deeper into the soil profile (Weaver 1926; Ferrant & Sprague 1940; Lamba et al. 1949; Niemi et al. 2005). This allows for the exploitation of different soil layers to recycle and retain nutrients and water.

As legumes, these species can fix nitrogen in the soil, which can enhance nitrogen availability for subsequent crops and improve yields (Vance 1998; Ross et al. 2009; Riesinger & Herzon 2010; Parr et al. 2011; Jani et al. 2016; Moore et al. 2020; Yang et al. 2020; MacMillan et al. 2022; Weinert et al. 2023; Andersen et al. 2025).

2.2.2 Melilotus officinalis

Melilotus officinalis, also called Yellow sweet clover, is utilized for many different purposes, such as phytoremediation, medicinal uses, fodder, green manure, potential fiber production, and nectar for pollinators (Turkington et al. 1978; Parrish et al. 2005; Brandsæter et al. 2008; Rigal et al. 2016; Kara 2021; Sowa-Borowiec et al. 2025). In this study, yellow sweet clover was primarily selected for its root system, biological nitrogen fixation (BNF), and winter hardiness.

Yellow sweet clover is a long-day biennial; it typically remains vegetative during the first year and flowers in the second. Its winter survival is influenced by sowing date and specific cultivar (Klebesadel 1992; Brandsæter et al. 2008). There are records of it acting as an annual, however, this is generally considered a rarity (Stevenson 1969). One of the main morphological advantages of this species is its deep taproot, which allows it to reach deeper into the soil than most other species in the flowering mix (Weaver 1926). Consequently, because it can survive the winter, it functions similarly to red clover as a “green bridge” over the winter, while annuals are cut down, this species have the ability to persist.

Furthermore, unlike many other legumes, this species occupies a broad ecological niche due to its ability to maintain viability in adverse soil conditions,

including waterlogged, high-salinity, and both alkaline and acidic environments. (Evans & Kearney 2003; Rogers et al. 2008; Rigal et al. 2016; Zhang et al. 2016; Dzyubenko et al. 2018). As a legume, it possesses the ability to fix atmospheric nitrogen through a symbiotic relationship with the bacterium *Sinorhizobium meliloti* (Sparrow et al. 1993; Harris et al. 2021). However, its use may be contentious; if left unchecked, it can drain the soil profile and reduce soil water content, thereby decreasing other plants' viability and inhibiting BNF (Wolf & Rohrs 2001; Wolf et al. 2004). This is why it is in a low amount in the flower mix for this study.

2.2.3 Lolium perenne

Lolium perenne, also called English ryegrass, is a versatile species utilized for grazing, hay, silage, cover cropping for soil improvement, lawns, turf, reducing soil erosion, and phytoremediation (De Baets et al. 2006; 2011; Nduwimana et al. 2007; Popay 2013). Key considerations for including English ryegrass in this mix are its robust root system and winter hardiness, which act as a "green bridge" (Cooper 1964; Xu et al. 2021) and can reduce nitrogen leaching and serve as a sponge for both water, nutrients, and biological activity (Norberg & Aronsson 2024).

English ryegrass is a perennial that must undergo vernalization before flowering; however, flowering often requires a long-day photoperiod to begin. Even though it can vary from different varieties (Cooper 1960; Aamlid et al. 2000; Wang & Forster 2017). Since this species is wind-pollinated, it doesn't provide nectar for pollinators as the other species in the mix do, but provides habitats for other insects, such as spiders *(Araneae* spp.*)* and different kinds of beetles *(Staphylinidae* spp.*, Carabidae* spp.*)* (MacLeod et al. 2004), which can provide different ecosystem services such as predation and seed consumption (Sunderland & Vickerman 1980). Besides its biological factor of hosting a habitat for ecosystem insects, it also has a dense root system, where it fibrous root system is concentrated in the top 0–20 cm of the soil profile, optimizing nutrient uptake in the active microbial zone, yet it can draw water from depths of 60–80 cm during dry periods (Garwood & Sinclair 1979).

Regarding subsidy regulations, English ryegrass isn't one of the approved species (Jordbruksverket 2026). Since English ryegrass's growth strategy can coexist with taller annuals without excessive competition during the establishment phase. It remains in the understory until the canopy is removed, after which it grows rapidly. With close contact with the Swedish Board of Agriculture, it was okay to use the English ryegrass in the mix, as long as it doesn't interfere with the flowering herbs[2].

[2] Lisa Karlsson, Environmental Analysis Unit, Swedish Board of Agriculture, mail, 2026-04-07

2.2.4 Raphanus sativus L. var. oleiformis Pers

Raphanus sativus L. var. *oleiformis Pers*, commonly known as Oilseed radish or fodder radish, has many different uses, ranging from biofuel, biochar, bran production, antimicrobial properties, and inhibition of weeds (Chammoun et al. 2013; Ricardo et al. 2018; Silva et al. 2020; Silvestre et al. 2020; Cavalheiro et al. 2021; Ncisana et al. 2024). For this study, this species was mainly chosen for its flowering, to support the subsidy, and secondly for its deep, thick root system.

Unlike winter rapeseed, oilseed radish develops a thick taproot (White & Weil 2011), which enables it to effectively penetrate compacted soil where fibrous root systems are unable to do so (Chen & Weil 2010; 2011). Research indicates that oilseed radish can increase the yield of the subsequent crop, as its roots utilize the pre-existing root channels (biopores) formed by the oilseed radish (Williams & Weil 2004; Chen & Weil 2011). This process potentially reduces the energy required to penetrate the soil and reach the subsoil faster.

In no-till systems, intercropping oilseed radish with a grass species is highly recommended, as the radish alone lacks the extensive fibrous root system and persistent mulch cover necessary for effective erosion control. (Głąb & Kulig 2008; De Baets et al. 2011). This is particularly important because no-till systems function differently from conventional tillage systems: while tillage buries biomass and leaves the soil bare and highly exposed to erosion, no-till systems receive erosion protection from surface biomass and root residue. However, a monoculture of a cover crop like radish lacks certain traits, meaning it cannot provide the same benefits as a mix of multiple plants with complementary characteristics. Thus, combining them increases erosion protection by ensuring both deep root structures and continuous ground cover. Beyond its ability to alleviate soil compaction, oilseed radish is exceptionally efficient at depleting nitrogen from deeper soil profiles, particularly in the form of nitrate (NO^{-3}), potentially reducing leaching (Thorup-Kristensen 2001; Kristensen & Thorup-Kristensen 2004; Sapkota et al. 2012; Wahlström et al. 2015; Elhakeem et al. 2023).

Flowering is regulated by both vernalization and photoperiod. Vernalization during early growth stages initiates flowering, although specific requirements for exposure time vary between different varieties (Jung et al. 2016). Furthermore, initiation is influenced by the photoperiod: the species typically exhibits an obligate vernalization requirement under short-day conditions, whereas vernalization is facultative under long-day conditions. Consequently, flowering can be initiated over long days without prior cold treatment, while it is inhibited under short days in the absence of vernalization. However, vernalization promotes flowering under both light regimes. (Erwin et al. 2002; Kaymak & Güvenç 2010; Han et al. 2026). Under Swedish conditions, characterized by long photoperiods, the plant will naturally initiate flowering and bolting, thus fulfilling the subsidy

requirements and die under frost conditions (Liu et al. 2013; Rodríguez-Gasol et al. 2026)

2.2.5 Fagopyrum esculentum

Fagopyrum esculentum, also called common Buckwheat, is a versatile crop with several agricultural and environmental applications. It serves as an alternative gluten-free grain, facilitates phytoremediation of aluminum (Al) and lead (Pb), and offers potential medicinal uses. Furthermore, it is efficient in low-input systems, induces resistance to clubroot diseases in *Brassica,* and mobilizes phosphorus (P) (Amann & Amberger 1989; Ma et al. 1997; Zheng et al. 1998; Tamura et al. 2005; Teboh & Franzen 2011; Possinger et al. 2013; Reyna-Llorens et al. 2015; Zhu et al. 2015; Lei et al. 2017; Habtemariam 2019; Domingos & Bilsborrow 2021; Thakur et al. 2021; Zhu 2021; Wu et al. 2025). In this study, the main criteria were its flowering characteristics and, partly, its fast-growing pattern for weed suppression (Eroğlu et al. 2024; Lami et al. 2024).

The initiation of flowering in common Buckwheat varies across ecotypes, with some adapted to long-day and others to short-day conditions (Takeshima et al. 2022). Flowering begins at the apical meristem and continues through lateral growth, generating continuous flowering (Grimes et al. 2025). Common Buckwheat is generally considered a facultative short-day plant; shorter days trigger earlier flowering, while longer days extend the flowering period and increase flower abundance (Michiyama et al. 2003; Quinet et al. 2004; Hara & Ohsawa 2013). The flowers have proven to have a beneficial effect on different insects, such as hoverflies *(Syrphidae* spp.*),* honey bees *(Apis mellifera)* and ladybugs *(Coccinellidae* spp.*)*, that provide ecosystem services including pollination and predation (Quinn et al. 2017; Rosado et al. 2021; Lami et al. 2024; Rodríguez-Gasol et al. 2026), which is most probably connected to its nectar (Patt & Rohrig 2017).

In a Swedish context, common Buckwheat is suitable for its flowering potential; however, its frost sensitivity necessitates later sowing to ensure successful establishment (Domingos & Bilsborrow 2021; Rodríguez-Gasol et al. 2026). Higher temperatures can increase flower production without reducing pollen content (Aubert et al. 2020), but when high heat is coupled with drought, pollen production may decrease (Aubert et al. 2021).

Beyond climatic factors, mild water stress has been shown to stimulate increased root length (Jamwal et al. 2015). Common Buckwheat has a favorable root-to-shoot ratio (Singh et al. 2025), producing a high shoot biomass relative to low root biomass, maximizing the mulching effect. The root system can reach a cumulative length of 1–2 m (Lei et al. 2025) and penetrate depths of at least 60 cm; however, the highest root density is found within the top 0–15 cm of the soil and increases macroporosity (Hudek et al. 2022).

2.2.6 Phacelia tanacetifolia

Phacelia tanacetifolia, also known as Lacy Phacelia, differs from the other species in the mix by having fewer commercial applications and is primarily used as an ornamental plant or a cover crop. In this experiment, the main criterion was the flowering. It was also chosen, like common Buckwheat, for its fast-growing pattern to effectively suppress weeds (De Baets et al. 2011; Liu et al. 2015; Kubíková et al. 2022b; Rodríguez-Gasol et al. 2026).

One of the main benefits of this species is its continuous flowering pattern, which benefits insects by providing a reliable nectar source for a longer period of time (Bowie et al. 1995; Giovanetti et al. 2022; Kubíková et al. 2022a). The flower attracts a wide range of insects from various families, mostly from Bumblebees *(Bombus* spp.*)*, honey bee *(Apis mellifera)* and wild bees/sweet bees *(Halictdae* spp.*)*, but also natural predators such as hoverflies *(Syrphidae* spp.*)* and green lacewings *(Chrysoperla carnea)* (Candelaria-Morales et al. 2022; Giovanetti et al. 2022; Rodríguez-Gasol et al. 2026). In honey production, the plant can play a big role in providing a larger yield of honey, due to its nectar and prolonged flowering (Giovanetti et al. 2022). Due to its frost sensitivity, the plant usually dies during the winter in Sweden (De Baets et al. 2011).

Liu et al. (2013) observed that, although Lacy Phacelia possesses a fine, relatively hollow root system with low biomass, this does not limit the rapid development of its aboveground biomass. Often resulting in outcompeting other weeds and plants.

2.3 Nitrogen dynamics and agricultural practices

In recent years, many farmers have increasingly adopted no-tillage or reduced-tillage practices to improve soil health. While plowing aerates the soil, it also accelerates mineralization and the reduction of organic matter (Stenberg et al. 1999; Bot & Benites 2005; Berntsen et al. 2006; Myrbeck et al. 2014), especially in vegetable-producing soils, where multiple harvests tend to lead to greater soil disturbance than in cereal-production soils (Jackson et al. 2004). However, ploughing is not merely for soil preparation; it also acts as weed control (MacLaren et al. 2021). Thus, in non-tillage or reduced tillage, weed management becomes more challenging. Leading many conventional farmers to rely on herbicides to combat weeds.

Soil is a highly complex system in which soil quality is influenced by various agricultural practices, including crop and fertilizer choices, the timing of different practices, climate, and soil type. Where both the chemical, physical, and biological parameters are interacting with each other (Maurya et al. 2020). Therefore, to optimize the use of arable land, it is essential to evaluate whether

glyphosate (non-tillage) differs from plowing (tillage) in terms of N plant availability and biological activity with or without flowering field.

Planning soil management practices involves considering multiple factors, including soil type, environmental conditions, common weeds, and crop rotation (MacLaren et al. 2021).

2.3.1 Nitrogen flow in the agricultural system

Nitrogen is a major factor in the agricultural system and can be derived from organic or inorganic sources, and is metabolized into different forms for the plant to take up. Where nitrate and ammonium are the most common inorganic forms, and more complex forms derive from the organic (Mokhele et al. 2012; Wang et al. 2025). However, the agricultural production system can alter the dynamics and flow of nitrogen, with the major factor being the external addition of different fertilizers (Petersen et al. 2023).

Soil management and nitrogen leaching

Soil management practices, such as mouldboard ploughing, reduced tillage, and no-tillage, influence nitrogen (N) flows and soil parameters. Research highlights a trade-off between mitigating nitrogen leaching and maintaining soil structure, a balance that is highly dependent on soil texture and regional climate (Myrbeck et al. 2014; Norberg & Aronsson 2024). In many scenarios, precipitation levels and clay content act as the primary drivers of nitrogen leaching rather than the tillage method itself; higher precipitation increases leaching, whereas increased clay content typically reduces the risk (Norberg & Aronsson 2025). Similar dynamics have been reported under Finnish conditions on soils with comparable clay content. In these systems, reduced tillage was shown to decrease nitrate (NO_3^-) leaching and soil erosion, particularly during the autumn, affecting both surface runoff and drainage flow (Koskiaho et al. 2002).

The timing of soil management is also a critical factor for nitrogen dynamics. Myrbeck et al. (2014) observed that in soils with lower clay content (28–43%), delaying tillage until late autumn resulted in lower soil mineral nitrogen (SMN) and reduced subsequent nitrogen leaching compared to earlier mouldboard ploughing. In contrast, on heavy clay soils (58–79%), delayed tillage had negligible effects on nitrogen leaching. However, late-season management when soil water content was high led to the formation of larger soil aggregates; although this increased aggregation was temporary and lost during winter freeze-thaw cycles, repeated late-season ploughing over several years was found to reduce yields (Myrbeck et al. 2012; 2014). This suggests that soil practices can have cumulative negative effects over time. Finally, combining shallow stubble cultivation with deeper mouldboard ploughing has been shown to significantly

increase SMN levels compared with mouldboard ploughing alone (Myrbeck et al. 2014).

Soil management practices often yield different results in sandy soils than in clay soils due to their higher drainage capacity. Research indicates that spring tillage can effectively reduce nitrogen leaching during the rain period (Norberg & Aronsson 2024). This is supported by Stenberg et al. (1999), who found that the timing of cultivation is crucial: early mouldboard ploughing led to a higher accumulation of SMN than late plowing, likely due to increased mineralization rates in the earlier-disturbed soil.

The impact of management intensity was also evident in a Danish study on sandy dairy farm soils (85–87% sand). Berntsen et al. (2006) observed that spring ploughing of a three-year-old grass-clover ley with urine application increased both SMN and nitrogen leaching, compared to keeping the field unploughed as a grazed ley for another year. This surge can probably be attributed to the rapid mineralization of accumulated organic nitrogen and carbon, further accelerated by the added urine. Additionally, intensive autumn management, such as harrowing three times, was shown to further increase SMN levels in some fields and subsequent nitrogen leaching risk. Furthermore, long-term monitoring in Denmark has demonstrated that while reduced nitrogen input generally leads to lower leaching, soil texture remains a primary variable. Sandy soils exhibit a greater propensity to leach residual nitrogen post-harvest than loamy soils. Additionally, the study showed that leaching levels vary by farm type, with distinct differences observed between pig, dairy, and arable (non-livestock) farms (Blicher-Mathiesen et al. 2014)

Nitrogen Emission agricultural system

Nitrogen can also be lost to the atmosphere through denitrification, primarily as dinitrogen gas (N_2) and nitrous oxide (N_2O). While cover crops generally increase soil organic carbon (SOC), their impact on N_2O emissions is highly species-specific. Research indicates that the choice of cover crop species significantly influences the magnitude of these emissions, as different species exhibit varying potentials for N_2O release (Olofsson & Ernfors 2022; Andersen et al. 2025).

Soil texture and seasonal variations further drive these gaseous dynamics. Skiba & Ball (2002) observed that while clay soils tend to emit more N_2O due to higher moisture and bulk density in autumn, sandy soils generally produce higher levels of NO. However, these emissions are not static; they exhibit seasonal fluctuations. In sandy soils, NO emissions peak when soil temperatures are higher during the autumn months. Despite these texture-specific baselines, both N_2O and NO emissions increase dramatically across all soil types following fertilization, representing temporal spikes.

These "hot moments" are further discussed by Zentgraf et al. (2025), who conclude that N_2O spikes in sandy soils are triggered by the complex interaction of soil heterogeneity, fertilization, and heavy rainfall. Management practices also influence the temporal dynamics of these losses. Krol et al. (2016) found that while ploughing triggers rapid but short-term N_2O pulses, reduced tillage results in a slower but more sustained increase in emissions. This prolonged emission in reduced tillage systems may be linked to the promotion of fungal communities, which play a significant role in N_2O-derived denitrification (D'Hose et al. 2016; Jia et al. 2025).

Cover crops effect on nitrogen leaching

Two Nordic reviews conclude that the impact of cover crops on nitrogen leaching is highly dependent on both species selection and soil texture. For instance, while grasses on sandy soils effectively reduce nitrogen leaching, red clover on clay soils may instead increase it; furthermore, a trade-off is often observed where the yield of the subsequent crop is reduced (Aronsson et al. 2015; Valkama et al. 2015). However, these effects are not always uniform. Stenberg et al. (1999) found that perennial grasses did not significantly affect N leaching in certain cases, likely due to variations in ploughing timing and weed competition.

The management and longevity of the cover crop also play a vital role. While cover crops can improve aggregate stability in loamy soils, even when autumn-ploughing (Breland 1995). Regarding tillage timing, Norberg & Aronsson (2024) observed that the lowest nitrogen leaching, in sandy soil (82%), occurred with spring tillage combined with a catch crop, though the effectiveness of the catch crop in reducing nitrogen leaching tended to decrease over time. Different cover crops in a sandy soil (83%) reduced the nitrogen leaching, and if legumes are in mixes, they leach less than in monocultures (Elhakeem et al. 2023). Additionally, the distinction between crop lifecycles is significant, as annual cover crops generally exhibit higher nitrogen leaching compared to perennial varieties (Norberg et al. 2022).

Glyphosate's effect on nitrogen and microorganisms in the agricultural system

As mentioned earlier, non-tillage can increase the demand for herbicides because weed control from ploughing is nonexistent. A recent report shows that, among all pesticides, herbicides account for roughly 80% of pesticide use in Sweden for vegetable, fruit, and cereal production (Melberg 2026).

Kepler et al (2020) observed that glyphosate doesn't significantly influence the microbial community structure. Instead, farming systems (e.g. tillage practices) and geographic location were the primary drivers of the community composition. Furthermore, when comparing community richness, no evidence was found that glyphosate had a significant impact, as changes occurred on both glyphosate-

treated and untreated plots. This indicates that seasonality has a greater impact on microbiome richness than glyphosate. Some microorganisms can degrade glyphosate and use it as a food source, and the degradation mechanism can vary among them (Shinabarger & Braymer 1986; Dick & Quinn 1995; Singh & Walker 2006; Ermakova et al. 2017; Firdous et al. 2020). This can partly be explained by the findings of Nivelle et al. (2017), who observed increases in NO_3^- and PO_4^{3-} following glyphosate application. This effect was particularly pronounced at extreme doses (100 times the field rate), where unfertilized soil (with no nitrogen fertilization for six years) showed a greater relative increase in nutrient availability compared to previously fertilized soil. These results suggest that a long-term history of nitrogen fertilization can alter the soil microbiome's response to glyphosate exposure, at least in a controlled, plant-free environment. Interestingly, Nivelle et al (2018) saw that glyphosate without nitrogen gave a significantly higher concentration of arbuscular mycorrhizal fungi (AMF) than glyphosate with nitrogen. They also found that glyphosate with nitrogen gave a higher total nitrogen in the soil than unfertilized soil, and the same pattern was observed in average net nitrification. Generally, they observed that if a soil wasn't given nitrogen, it increased various microbial activities, regardless of whether glyphosate was applied. As for nitrogen and glyphosate, there was a reduction in these two when they were applied together in microbial activity.

2.3.2 Effects of soil management on carbon dynamics

Increasing soil organic matter (SOM) is a long-term process, as it comprises both labile (fast-cycling) and recalcitrant (slow-cycling) carbon pools with turnover times ranging from years to decades. In technical terms, SOM refers to all organic components in the soil, while SOC is the measurable carbon fraction of that matter (roughly 58%). Detecting significant increases in SOC due to specific management practices typically requires more than a decade (Soon et al. 2007; Spiegel et al. 2025a)

However, the microbial component of the soil is much more sensitive to management changes than the total carbon pool. Measuring microbial biomass and activity provides an "early warning" indicator of soil health, as microorganisms are the primary drivers of carbon cycling, they transform fresh residues into stable SOC. Less soil disturbance generally leads to higher microbial biomass and AMF in the topsoil (0–10 cm), as seen in reduced tillage systems (D'Hose et al. 2016; Spiegel et al. 2025b).

Furthermore, soil management significantly alters the vertical distribution of biological activity. While non-tillage concentrates microbial biomass and earthworm populations in the surface layer (0–10 cm), mouldboard ploughing redistributes organic matter, leading to increased microbial biomass and carbon in the 10–30 cm profile (Mutegi et al. 2013; Spiegel et al. 2025b). This vertical

differentiation is crucial for soil sampling strategies, as organic matter and microbial activity may shift below the sampling depth depending on the tillage intensity. D'Hose et al. (2016) also found that different practices altered the concentrations of bacteria and fungi, noting that earthworm populations increased significantly only with reduced soil disturbance or the addition of compost.

2.4 Soil health indicators

Soil health has, over the recent years, been a hot topic and has thus been quite diffuse, as many people take the definition quite loosely and use it as an umbrella term. However, both the USA and the EU have come up with a definition where they share the same meaning:

> "'Soil health' means the physical, chemical and biological condition of soil, determining its capacity to function as a vital living system and to provide ecosystem services (The European Parliament and the Council of the European Union 2025)."

> "The continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (USDA NRCS 2026)."

Since the subsidy for flowering fields is mainly based on improving the biodiversity through flowering and different insects, it doesn't touch upon the synergetic effect it can have on the soil. This study aims to better understand the impact flowering fields have on the soil and, hopefully, benefit both by improving biodiversity from the flowering and increasing overall soil health.

Soil measurements can be categorized into three parameters: biological, chemical, and physical (Maurya et al. 2020). The same review concludes the importance of different analyses varies depending on the type of soil to be measured. Previously, many measurements were focused on chemical (nutrient) and physical (soil properties), since they provided a good indication of yield. It has recently been important to see how the biological aspect correlates with the analysis, alongside the chemical and physical parameters. This is why the Cornell soil health laboratory's comprehensive assessment of soil health standard operating procedures (CASH) (Schindelbeck et al. 2016) was developed to assess all three parameters and assign them a CASH score based on multiple analyses and thus quantifying soil health.

This study aims to examine the balance between nitrogen and the biological processes associated with different soil practices and crops. To get a snapshot of chemical measurement, Spurway analysis was taken to analyze the plant available ammonium, nitrate, and pH value. From the same soil sample, microbial analysis was performed, including microbial activity (FDA, Fluorescein Diacetate

Analysis), soil respiration, active carbon (POXC), and soil protein (ACE, Autoclaved-Citrate Extractable). For the physical aspect, SOM were measured.

3. Experiment

3.1 Data sampling

The literature review was used by searching for relevant keywords regarding the text subject. This was done by searching in different databases, including Scope, Google Scholar, and Primo.

To refine the academic English and structure of the paper, the generative AI tool Gemini 3 Flash was used as a language editor. The original drafts, scientific arguments, and selection of literature were developed by the author. The AI was used iteratively to improve clarity and flow, informed by the author's specific instructions and corrections.

3.2 Materials and methods

3.2.1 Experiment layout

The experiment was conducted in the south-west of Scania, close to Löddeköpinge (Longitude: 55.748701, Latitude: 13.029622), in a maritime climate. The field is managed by the advising firm HIR Skåne, according to conventional practices in Sweden, including the use of pesticides and mineral fertilizers as well as various soil management practices, such as ploughing/harrowing. Soil analysis according to Ammonium Lactate Extraction (AL) was pH 7.3, P 25, K 6.3, Mg 5.6 K/Mg-ratio 1.1 and Ca 176. Soil fraction analysis was also conducted by HIR Skåne and yielded 2.2% organic matter, 6% clay, 10% silt, and 82% sand, indicating a coarse soil (between loamy sand and sand) according to Moebius-Clune (2016). Before the experiment started, spring barley was plowed down before winter, and the soil was left fallow over winter.

Each plot was 9 × 9 m, and each treatment was replicated six times, resulting in 24 parcels (See Appendix 2 for a visual representation).

3.2.2 Management of the experiment:

Twelve units were sown with spring barley, and oilseed radish was sown in after harvest (Control). The remaining twelve cells were sown with the flowering field mix. Within each set of twelve units, six were ploughing, and six were sprayed with glyphosate in the spring before planting Celeriac *(Apium graveolens* var. *rapaceum)* in all plots. The flowering field mix received 40 kg N^{-1} at sowing, and the control received 100 kg N^{-1} at sowing the spring barley (Appendix 1; table 1, for detailed dates and management practice).

Soil samples were collected five times during 2025 and 2026. The first sample was taken as a baseline for nutrient status, and the second was taken a few weeks before the first frost. The third sampling was taken several weeks after the frost,

and the fourth and fifth sampling dates were taken before and after glyphosate and ploughing application (Appendix 1 for detailed dates and management inputs). This was done to see how the soil responded to different external factors across species in the plots. Since half of the plots were sown with oilseed radish which dies of during the winter, and the flowering field had some species that survived the winter, the study aimed to examine nutrient profiles and different microbial aspects. The same idea was intended for the ploughing and sprayed plots.
From each plot, eight soil cores sticks were taken from 0-30cm deep with a soil auger (Ø 2.7cm) and rigorously mixed in a bucket before being poured into a plastic bag. All the samples were labeled with the corresponding plot and then placed in a cold chamber (+1/+8 °C) for storage. Three Falcon tubes (50 mL) from each replicate sample were filled and frozen for future analysis.

The Flowering field mix comprised eight species, including annuals, biennials, and perennials (Table 1). The ratio was derived from discussions with the seed company Olssons Frö and HIR Skåne advisers (Appendix 3).

Table 1: Composition of the flowering field mix and the control.

Flowering field mix:	Control:
Red clover *(Trifolium pratense)*	Spring barley *(Hordeum vulgare L.)*
Crimson clover *(Trifolium incarnatum)*	Oilseed radish *(Raphanus sativus L.* var. *oleiformis* Pers*)*
Persian clover *(Trifolium resupinatum)*	
Sweet clover *(Melilotus officinalis)*	
Lacy Phacelia *(Phacelia tanacetifolia)*	
Buckwheat *(Fagopyrum esculentum)*	
Oilseed radish *(Raphanus sativus* L. var. *oleiformis* Pers*)*	
English rye grass *(Lolium perenne)*	

3.2.3 Analysis of soil biology

For all soil samples, fresh soil was collected the day before the analysis day and air-dried overnight. On the analysis day, it was then sieved through a 2mm sieve.

All analyses were performed according to the protocol of Schindelbeck et al. (2016) except for the Fluorescein Diacetate Analysis (FDA), which was performed according to Green et al. (2006).

Active carbon (Permanganate Oxidizable Carbon, POXC)

Air-dried soil was weighed to 2.5g and placed in a 50 ml aluminum-covered Falcon tube. 18 mL of distilled water was added, and 2 mL of 0.02 M potassium

permanganate ($KMnO_4$) solution was added to the Falcon tube to initiate the reaction. One control was added without the soil. It was shaken for 2 minutes at 120 rpm (0025002982, HS 501 D, IKA, Staufen im Breisgau, Germany). After 2 minutes, the falcon tubes were placed on the counter for 8 minutes to allow the soil to sediment. After 10 minutes of reaction time, 0.5 mL of the aliquot was transferred to a new Falcon tube containing 49.5 mL of distilled water, and the mixture was shaken for 10 seconds. 0.1 ml was then transferred to cuvettes and placed in a spectrometer and read at 550 nm (LPV440.99.00001, DR3900, Hach, Dusseldorf, Germany).

The content of Active Carbon (Permanganate Oxidizable Carbon, POXC) was determined by measuring the reduction in the purple color of a potassium permanganate ($KMnO_4$) solution. The final value was calculated using a linear regression from a standard curve and the following equation:

$$Active\ C\ mg^{-1}kg^{-1} = [0{,}02 - (a + b * Abs)] * 9000 * \frac{V}{m}$$

Abs: The absorbance value of the sample at 550nm.
a and b: The intercept and slope, respectively, derived from the linear regression of the standard curve.
0.02: The initial concentration of the $KMnO_4$ solution before reaction (mol/L).
9000: The constant representing the milligrams of carbon oxidized by one mole of $KMnO_4$ is reduced (mg C/mol).
V: The volume of the $KMnO_4$ solution used, expressed in liters (0.02 L).
m: The dry mass of the soil sample, expressed in kilograms (0.0025 kg).

Autoclaved-Citrate Extractable (ACE) Soil Protein

Air-dried soil was weighed to 3.0g and placed in a 100 mL glass bottle with 24 mL of sodium citrate buffer (20 mM, pH 7.0). It was shaken for 5 minutes at 180 rpm (0025002982, HS 501 D, IKA, Staufen im Breisgau, Germany). After mixing, the bottles were autoclaved for 30 minutes at 121 °C and 15 psi, then allowed to cool. 2 mL was then collected and added to a microcentrifuge tube, and the mixture was centrifuged at 10.000g. 0.8 mL of the Aliquots from the centrifuged sample was then pipetted to new microcentrifuge tubes without collecting the bottom fractions. Then, the BCA (Bicinchoninic Acid, 23227, Pierce BCA Protein Assay Kit, Thermoscientific, Rockford, IL, USA) working reagent was mixed with 19 mL of Regent A and 0.380 mL of Regent B. This mixture was placed in a 96-well microplate with 0.2 mL in each well. 0.1 mL of the soil sample and standards (23209, Albumin Standards, Thermo Scientific, Rockford, IL, USA) was pipetted into the microplate, and each replicate was performed twice. A plastic film was affixed to the microplate and incubated at

61,5 °C for 60 minutes. After the incubation time, the sample was placed on the counter to cool for 10 minutes, then the plastic film was carefully removed and placed in a spectrophotometer (A51119700, Mulitskan, SkyHigh, Thermoscientific, Singapore), and the sample was measured at 562nm.

For each analysis, a standard curve was established using quadratic regression. The mean absorbance value from the soil samples was used to determine the extract concentration (C_{ext}) by solving the quadratic equation. The final ACE soil protein was then calculated using the following equation:

$$P_{soil} = \frac{C_{ext} * V}{m}$$

P_{soil}: Protein concentration (mg protein per g dry soil).
C_{ext}: Protein concentration in extract (mg/L), derived from the quadratic regression of measured standard curves.
$C_{ext} = \frac{-b+\sqrt{b^2-4a(c-Abs)}}{2a}$
V = total volume of the extraction solution (mL).
m = Mass of the dry soil sample (g).

Soil respiration

Air-dried soil was weighed to 20.00g, and placed in an aluminum form with 9 pin-holes in the bottom. 1 Whatman filter paper (Ø 70mm) was placed in a 500 mL glass jars, and 7.5 mL of distilled, deionized water was transferred to the paper in the bottle. The soil-filled aluminum form was then placed on top of the filter papers. A contraption of chicken wire was constructed to stand in the glass jars, with a petri dish attached to the tops. A Petri dish was mounted on a glass vial fitted with tape; the bottle was then filled with 9 mL of 0.5 M potassium hydroxide (KOH). The glass jars were sealed with a lid and incubated at room temperature for 4 days. After incubation, the lid was opened, and the EC was measured in the KOH solution (HQ440D.98.00012, HQ440d multi conductivity meter, Hach, Düsseldorf, Germany). The respiration (mg CO_2-C kg^{-1} day^{-1}) was then calculated as follows:

$$Respiration = \frac{\left(\left(\frac{EC_{raw} - EC_{sample}}{EC_{raw} - EC_{sat}} - \frac{EC_{raw} - EC_{control}}{EC_{raw} - EC_{sat}}\right) * V_{KOH} * N_{KOH} * 22000\right) * \frac{12}{44}}{m * t}$$

EC_{sample}: The electrical conductivity of the KOH solution after soil incubation.
$EC_{control}$: The electrical conductivity of the KOH solution in a blank control (no soil).
EC_{raw}: The conductivity of the initial KOH solution.
EC_{sat}: The conductivity of a fully carbonate-saturated KOH solution.

V_{KOH}: The volume of the KOH trap solution used.
N_{KOH}: The normality (concentration) of the KOH solution.
22000: Conversion factor for mg CO_2 per equivalent.
12/44: Stoichiometric ratio used to convert CO_2 mass to carbon mass (C).
m: The dry weight of the soil sample in kilograms.
t: Incubation time in days.

Fluorescein Diacetate Analysis (FDA)

The soil was air-dried, then sieved through a 2mm sieve, and 1 g was transferred to a 100 mL bottles, where 50 mL of 60 mM sodium phosphate buffer (Na_3PO^4*$12H_2O$, pH 7.6) and 0.5 mL of 4,9 mM FDA lipase substrate solution ($C_{24}H_{16}O_7$ in reagent-grade acetone) were added. One control was prepared without soil, using 50 mL of 60 mM sodium phosphate buffer and 0.5 mL of acetone in a 100 mL glass bottle. Controls were also made for each replicate with representative soil, adding 50 mL of 60 mM sodium phosphate buffer and 0.5 mL of acetone in a 100 mL glass bottle. The bottles were sealed with the lids, swirled for a few seconds, and then incubated for 3 h at 37°C. After 3 h, 2 mL of reagent-grade acetone was added, and the mixture was gently mixed to terminate the FDA hydrolysis. 30 mL of the substrate suspension was transferred to a 50 mL centrifuge tube and centrifuged at 8000 rpm (8820 g) for 5 min. After centrifugation, the supernatant was filtered through Whatman No. 2 filter paper (Ø 150mm). Once the filtration was complete, 1 mL of the filtered solution was placed in a cuvette and inserted into the spectrophotometer (LPV440.99.00001, DR3900, Hach, Dusseldorf, Germany) at 490 nm to measure fluorescein. It is essential that the process be completed within 30 minutes of adding 2 mL of reagent-grade acetone to ensure maximum accuracy. The amount of fluorescein (mg/g soil) was calculated from the spectrophotometer absorbance at 490 nm using the standard curve of absorbance versus fluorescein concentration (mg). The total microbial activity was determined by the hydrolysis of fluorescein diacetate (FDA).

$$FDA\ (mg\ g^{-1}) = \frac{Absorbance\ -\ 0.029}{3.0269}$$

Absorbance: The optical density measured at 490 nm.
0.029: The y-intercept from the fluorescein standard calibration curve.
3.0269: The slope (k) of the standard calibration curve, representing the relationship between absorbance and fluorescein concentration.

Soil Organic Matter (SOM)

Approximately 30g of soil was taken into already weighed aluminum forms and then dried at 105 °C for 2 h or overnight. The soil samples were weighed again

and placed in an oven at 550 °C for 2 h, to see the loss on ignition (LOI) (weighing both the forms and the soil separately). After heating to 550 °C, the soil was weighed for the final time. The percentage of organic matter (%OM) was calculated by using the equation as follows:

$$\%LOI = \left(\frac{Weight_{before}\,550°C\ -\ Weight_{after}550°C}{Weight_{before}\,550°C}\right) * 100$$

$$\%OM = (\%LOI * 0.7) - 0.23$$

$Weight_{before}$ 550°C: The mass of the oven-dry soil sample before being placed in the muffle furnace.
$Weight_{after}$ 550°C: The mass of the soil sample after combustion at 550°C (only minerals/ash remain).
0.7 and 0.23: Regression coefficients used by Cornell University to correct for the loss of structural water in clay minerals, ensuring a more accurate estimate of actual organic matter.

3.2.4 Analysis of soil chemical properties

Spurway

Approximately 500-600g grams of fresh soil from each replicate was sent to LMI AB (Helsingborg, Sweden) for Spurway analysis. To measure nitrate, ammonium, phosphorus, calcium, magnesium, sulfur, boron, iron, sodium, and aluminum, the soil was extracted with acetic acid (CH_3COOH) for 30 minutes to simulate plant-available nutrients; the solution was then filtered and analyzed in a spectrophotometer (LMI N.D.).

To calculate nitrate and ammonium from the given samples of mg/L to kg/ha, the following calculation was made:

$$N_{stock} = \frac{C_{soil} * Area * depth}{k}$$

N_{stock}: Total nitrogen stock (kg N ha^{-1})
C_{soil}: Volumetric concentration of nitrogen in the soil (mg L^{-1})
Aera: Unit area (10,000 m^2, representing 1 hectare)
depth: Sampling depth (0.3 m)
k: Conversion constant (1,000) to convert grams to kilograms

3.2.5 Statistical analysis

The raw data were saved in Excel, and all statistical calculations were made in RStudio version 4.3.1 (2023-06-16). To see which program was used in R, look at Appendix 5.

The statistical analysis was performed on the difference between separate sampling occasions (*phase 1*; *before winter* and *after winter and phase 2; before treatment* and *after treatment*), rather than using raw values. This approach was chosen because the sampling dates were not continuous and soil properties vary widely due to changing biological processes over time, making a standard repeated-measures ANOVA difficult to apply. The difference was calculated to show the change over time between the two dates (*Date 2 – Date 1; Date 4 - Date 3*).

$$After - Before = Change$$

Phase 1 is based on one-way analysis of variance (ANOVA) between the different mixes (*Flower_mix* and *Oilseed_radish*). The same analysis was made for each block to see if the blocks are different from each other, since each block (a total of six blocks; Appendix 2) has one of each treatment.

Phase two is based on a two-way analysis of variance (ANOVA) between seed mix (*Mix*), management method (*Treatment: ploughed vs. spraying*) and the interaction of these (*Mix*Treatment*). Block was also included in this analysis as well.

The estimated mean (EM-mean) was then calculated from this difference, and a post-hoc Tukey HSD test ($p<0.05$) was used to assign significant letters. To know that the change is accurate with the starting value and ending value of the effect, the mean of the observed value was used to ensure that the change is correct and has a higher observed value, and not just a higher change value. To ensure that the assumptions for the analysis of variance (ANOVA) were met, Shapiro-Wilk normality tests and Bartlett's tests for homogeneity of variances were performed on the residuals of both models and phases. When the Bartlett test showed a significant result, Levene's test of homogeneity of variance was used as a robust method to deal with the block variance.

A HEAT-map was made to clarify which analysis is correlated based on color and quantification from -1 to +1 based on Spearman rank correlation matrix. Principal Component Regression (PCA) was made to get a holistic view of all the analysis and what two factors have the highest impact on the analysis.

3.3 Results

All the analyses except for nitrate and ammonium showed non-significant results for one-way ANOVA in phase 1.

3.3.1 Analysis of soil biology

Fluorescein Diacetate Analysis (FDA):

Phase 1:

A trend can be seen that Flower mix has a higher estimated mean of change that is almost significant between the seed mixes ($F_{1.17}$=3.9; P=0.062) and a higher observed value, post-treatment, than the oilseed radish (Figure 1: Appendix 6; Table 6).

A post-hoc Tukey HSD test confirmed that the increase in FDA activity was not significant in the mixes (Figure 1).

Phase 2:

Two-way ANOVA showed a significant effect from the seed mix ($F_{1.15}$ = 22,7; P =0.00025).

Post-hoc Tukey HSD test showed that oilseed radish with spraying was

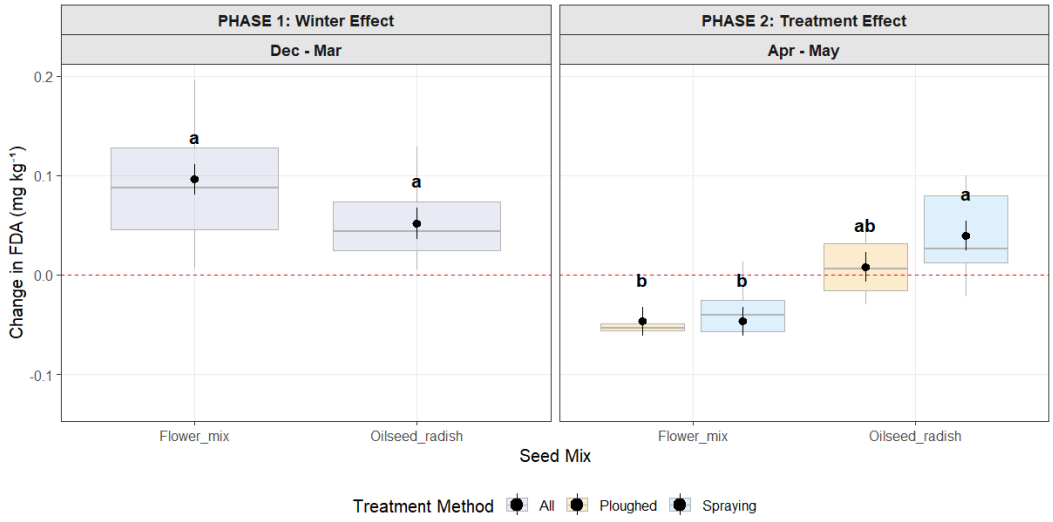


Figure 1: Change in FDA (mg kg^{-1}) during phase 1 (winter effect, Dec-Mar) and phase 2 (treatment effect, Apr-May) for different seed mixes and termination treatments. Boxplots show the distribution, while the black dots and error bars represent the mean ± SE. Different letter denote statistically significant differences within each phase according to Tukey's HSD test (p<0.005).

significantly different from both the flower mix treatment (figure 1; Appendix 6; Table 7). Only oilseed radish showed a net positive change in FDA activity (Appendix 6; Table 7). Oilseed radish with ploughed proved through post-hoc Tukey test HSD that it wasn't significant to other groups, sharing letter (ab) with all the other mixes and treatments.

ACE, Soil Protein

Phase 1:

Both seed mixes showed an increase in soil protein, with more protein (mg g-1) after the winter than before (Figure 2).

Phase 2:

For all treatments, there is a positive change in soil protein levels (Figure 2).

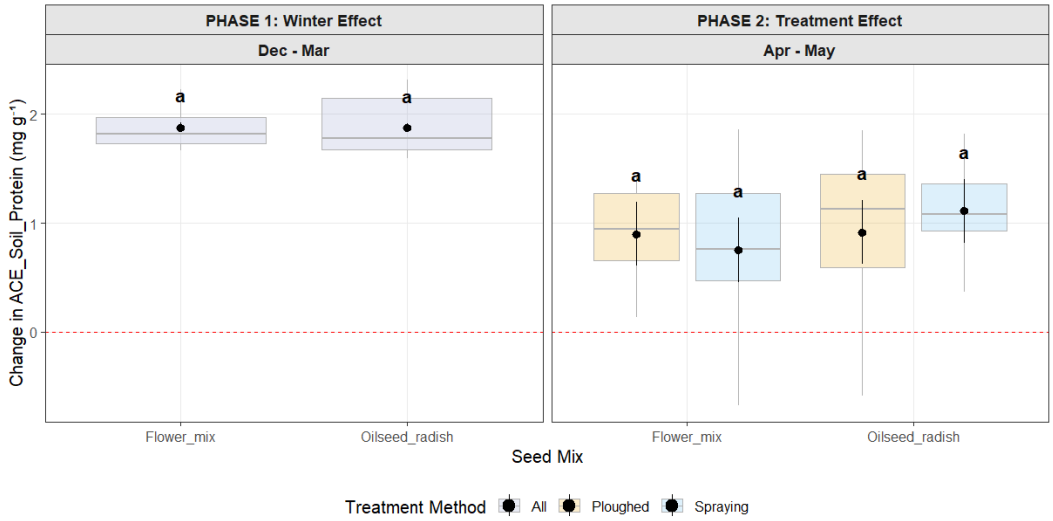


Figure 2: Change in ACE (mg g^{-1}) during phase 1 (winter effect, Dec-Mar) and phase 2 (treatment effect, Apr-May) for different seed mixes and termination treatments. Boxplots show the distribution, while the black dots and error bars represent the mean ± SE. Different letter denote statistically significant differences within each phase according to Tukey's HSD test ($p<0.005$).

Soil respiration

Phase 1:

Levene's test showed no significant difference (P=0.07). Respiration showed low change over the winter for CO_2-C respiration, for both mixes (figure 3).

Phase 2:

The highest change in respiration came from flower mix ploughed, which resulted in negative CO_2-C release from the soil, meaning that there was higher respiration before the treatment was applied (Appendix 6; Table 8). For the other mixes and treatments, there were no to low changes (figure 3).

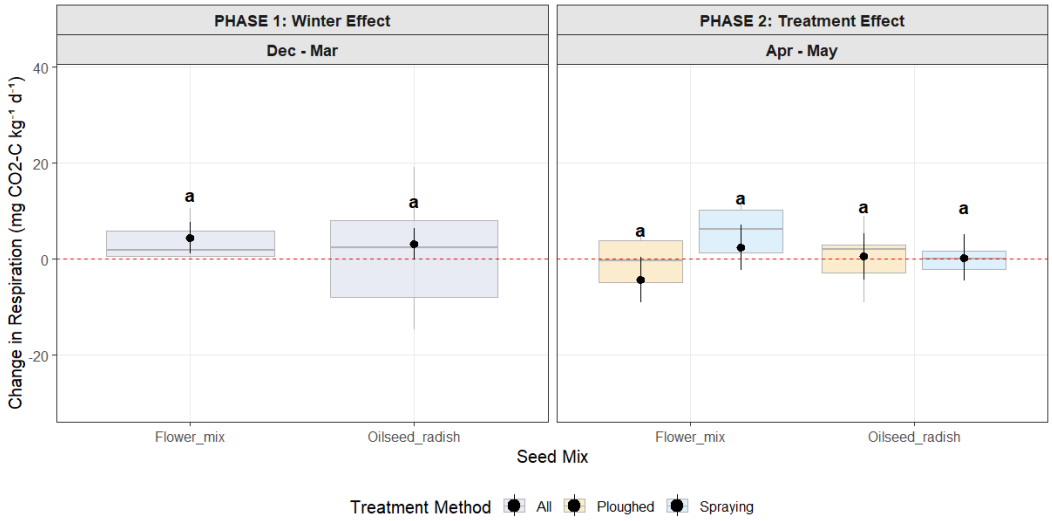


Figure 3: Change in Respiration (CO_2-C kg^{-1} d^{-1}) during phase 1 (winter effect, Dec-Mar) and phase 2 (treatment effect, Apr-May) for different seed mixes and termination treatments. Boxplots show the distribution, while the black dots and error bars represent the mean ± SE. Different letter denote statistically significant differences within each phase according to Tukey's HSD test ($p<0.005$).

Active carbon (Permanganate Oxidizable Carbon, POXC)

Phase 1:

The winter effect had a negative change in the POXC, resulting in a lower amount of soluble carbon (Figure 4). Flower mix had a higher amount before winter and resulted in a higher loss than oilseed radish, resulting in a higher loss of soluble carbon (Appendix 6; Table 9).

Phase 2:

A two-way ANOVA showed a significant effect of the treatment ($F_{1,15} = 6.13$; P=0.026). There was a slight trend towards the interaction between treatment and seed mix (*treatment*mix*), but not significant ($F_{1.15}$=4.37; P=0.053). The only significant level between the groups from the post hoc Tukey HSD test was between the flower ploughed and the flower sprayed.

The observed raw values before treatment and after treatment show that the flower mix with ploughed had the greatest change, despite treatments starting at approximately the same levels of POXC. Conversely, flower mix with spraying

was the only combination that showed a negative change (Figure 4; Appendix 6 table 7).

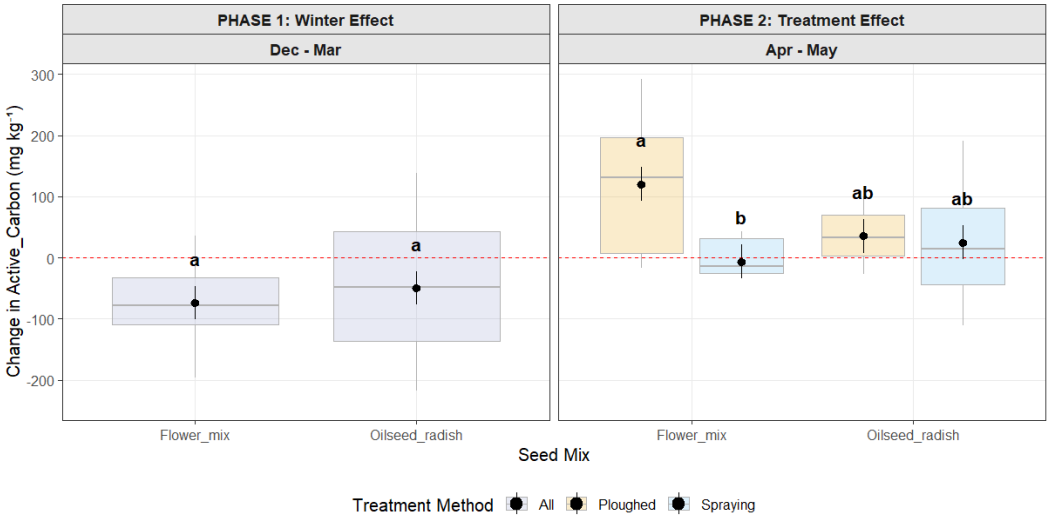


Figure 4: Change in Active Carbon (mg kg^{-1}) during phase 1 (winter effect, Dec-Mar) and phase 2 (treatment effect, Apr-May) for different seed mixes and termination treatments. Boxplots show the distribution, while the black dots and error bars represent the mean ± SE. Different letter denote statistically significant differences within each phase according to Tukey's HSD test (p<0.005).

3.3.2 Soil chemical properties

Nitrate

Phase 1:

Levene's test showed no significant difference (P=0.125). Winter effect showed a significant difference between the seed mixes ($F_{1,17}$=8.3; P=0.01). Post-hoc Tukey HSD test proved that the flowering fields exhibited significantly greater change in nitrate levels than oilseed radish after winter (Figure 5).

The observed values show that the baseline before the winter was the same for both seed mixes (Appendix 6; Table 11).

Phase 2:

Levene's test showed no significant difference (P=0.56). Oilseed radish had a higher amount of nitrate before the treatment than the flower field (Appendix 6; Table 12)

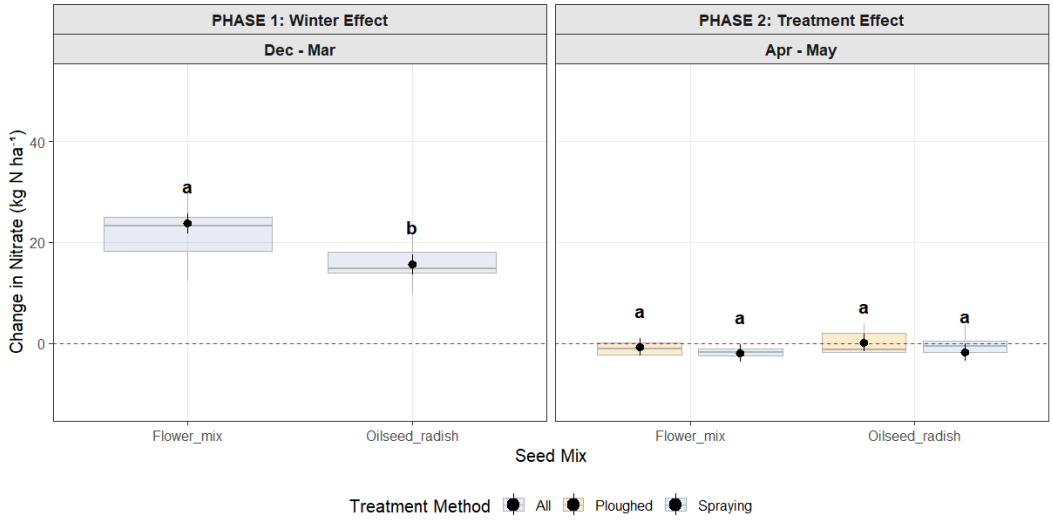


Figure 5: Change in Nitrate (kg N ha^{-1}) during phase 1 (winter effect, Dec-Mar) and phase 2 (treatment effect, Apr-May) for different seed mixes and termination treatments. Boxplots show the distribution, while the black dots and error bars represent the mean ± SE. Different letter denote statistically significant differences within each phase according to Tukey's HSD test ($p<0.005$).

Ammonium

Phase 1:

Levene's test proved non-significant (P=0.63), and the Shapiro-Wilk test proved significant (P=0.0007). The winter effect reduced the amount of soil ammonium (Figure 6).

Phase 2:

Two-way ANOVA showed no significance from either block, treatment, mix or interaction (*treatment*mix*). Interaction between treatment and mix (*treatment*mix*) was close to significant ($F_{1.15}$=4.22; P=0.057).

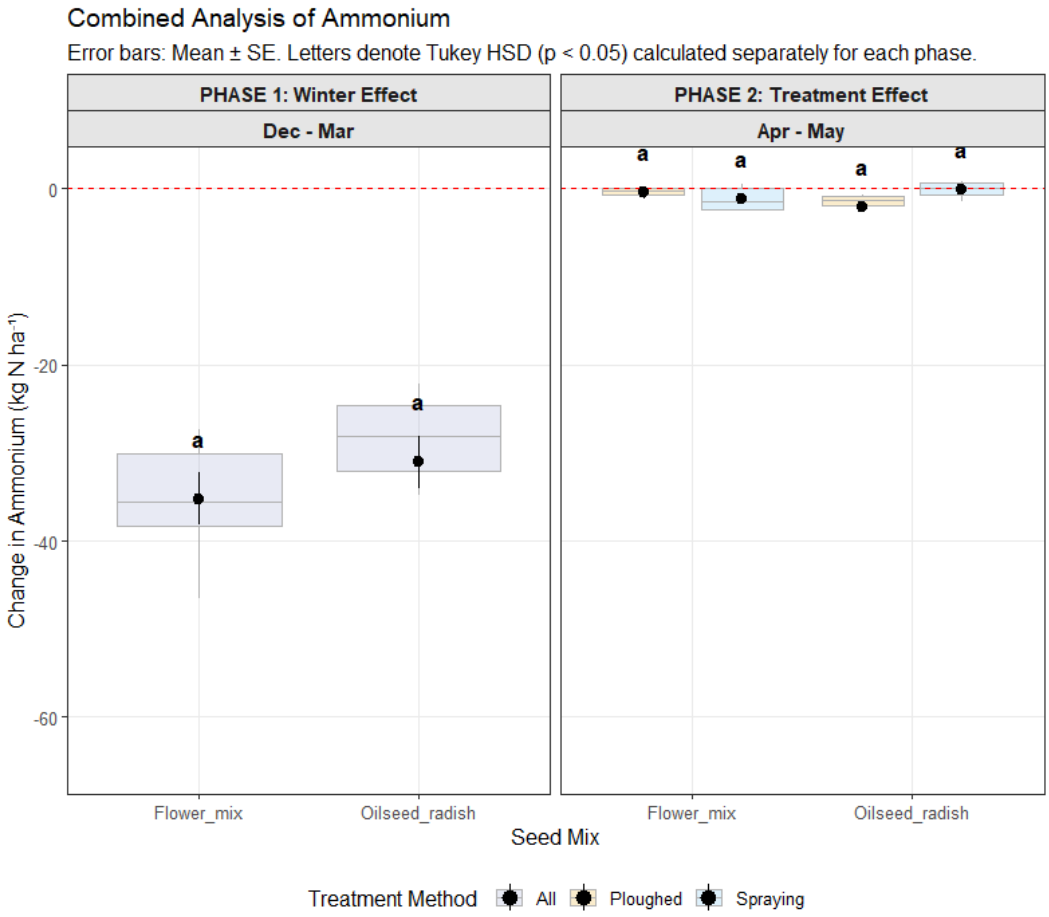


Figure 6: Change in Ammonium (kg N ha^{-1}) during phase 1 (winter effect, Dec-Mar) and phase 2 (treatment effect, Apr-May) for different seed mixes and termination treatments. Boxplots show the distribution, while the black dots and error bars represent the mean ± SE. Different letter denote statistically significant differences within each phase according to Tukey's HSD test (p<0.005).

SOM

Phase 1:

One-way ANOVA showed no significant effect from the block or seed mix over the winter.

Phase 2:

Shapiro-Wilk test showed significance (*P*=0.0003), and Levene's test showed non-significance (*P*=0.499).

Two-way ANOVA proved non-significant for the block and seed mix treatments.

pH

Phase 1:

One-way ANOVA proved non-significant over the winter. (Figure 7).

Phase 2:

Two-way ANOVA proved significant for the treatment ($F_{1,15}$=4.7; P=0.047). When a post-hoc Tukey HSD test was done, no significance between the groups was found (figure 7). A trend can be seen in the treatment effect, where spraying generally led to a positive pH change, whereas ploughing resulted in a lower or negative pH change, regardless of the seed mix s(figure 7).

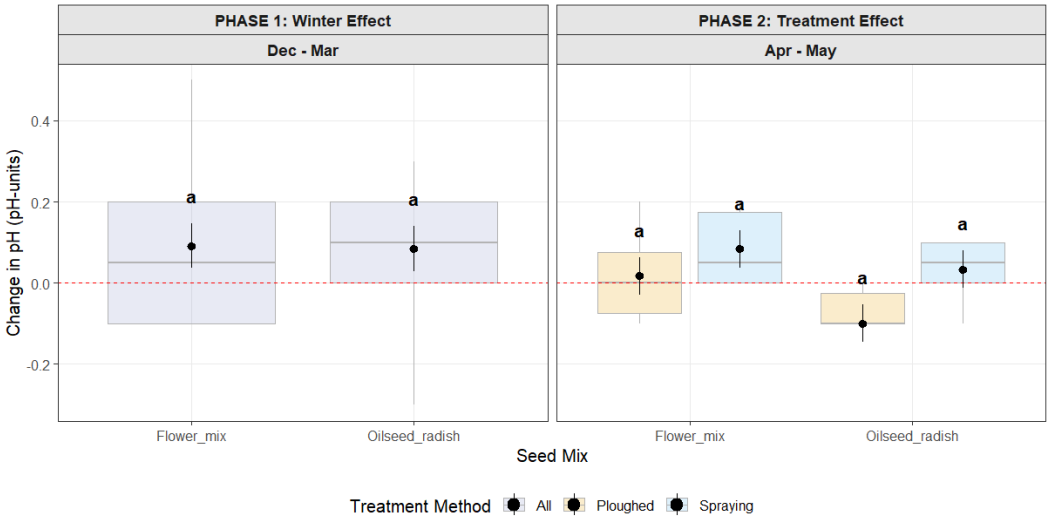


Figure 7: Change in pH (pH-unites) during phase 1 (winter effect, Dec-Mar) and phase 2 (treatment effect, Apr-May) for different seed mixes and termination treatments. Boxplots show the distribution, while the black dots and error bars represent the mean ± SE. Different letter denote statistically significant differences within each phase according to Tukey's HSD test (p<0.005).

3.3.3 PCA and HEAT-MAP

Principal Component Analysis (PCA)

The main effect is coming from the winter effect (PC1=45.05%), while the seed mix has a smaller effect (PC2=15.53%). The PCA for the winter effect displays that winter effect has an influence in ammonium-nitrate relation, where nitrate is higher after winter than before and reversed for the ammonium. It is also showed that the biological analysis ACE, FDA are closely related to the nitrate

after winter. For Ammonium and Active Carbon, it is higher before winter, than after winter (figure 8).

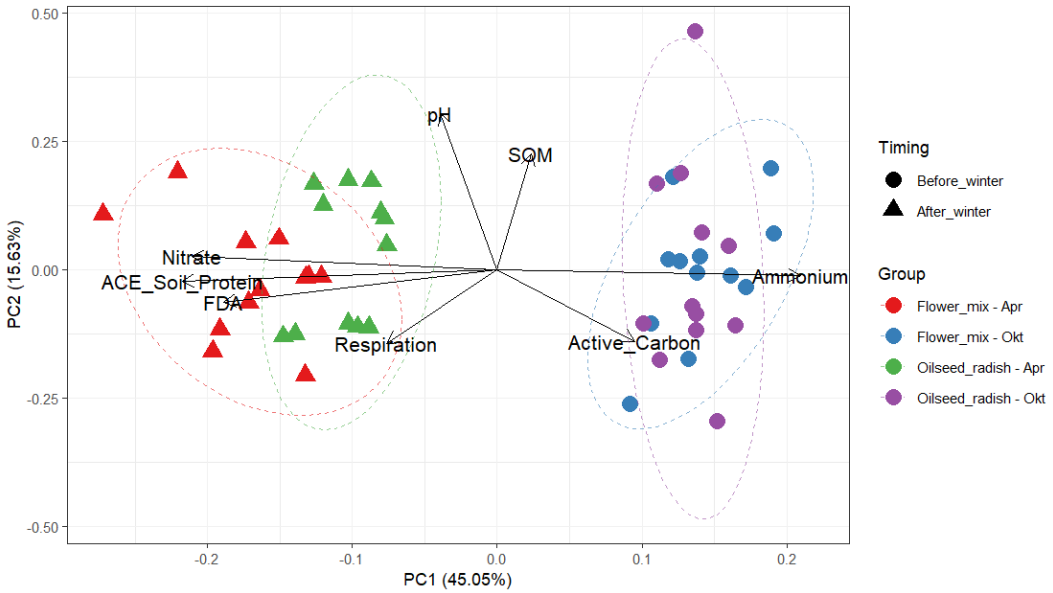


Figure 8: Principal Component Analysis (PCA) biplot of soil properties during Phase 1 (Winter Shift, Oct–Apr). The horizontal axis (PC1) explains 45.05% of the variance, and the vertical axis (PC2) explains 15.63%. Vectors (arrows) indicate the direction and strength of environmental variables. Individual sample points are shaped by sampling time (circles = before winter/October, triangles = after winter/April) and colored by treatment groups (Flower mix and Oilseed radish). Ellipses represent 95% confidence intervals for each group.

Spearman's Rank Correlation analysis (Heat-map)

Spearman's rank correlation revealed distinct seasonal and treatment shift in the relationship between soil properties in the two phases (Appendix 7; figure 10 and 11).

Phase 1:

Before winter, the flower field exhibited a strong negative correlation with nitrate and ammonium levels (r_s = -0.83), which disappeared post-winter (r_s =-0.02). In contrast, oilseed radish showed no initial correlation before winter r_s = 0.17) but developed a strong positive correlation after winter r_s = 0.70).

Other analyses highlighted relatively strong correlations both before and after winter for oilseed radish, particularly between ACE and nitrate/ammonium (r_s =0,.48 to 0,60). In the oilseed radish treatment, the correlation between respiration and SOM increased sharply from r_s = -0.17 before winter to r_s = 0.67. The flower field maintained a stable correlation across seasons (before winter: r_s =0.39; after winter r_s = 0.40), whereas oilseed radish shifted from a positive correlation (r_s =0.39) to no correlation r_s = 0.16). In the flower field, the correlation inverted

from negative before winter (r_s = -0.43) to strongly positive after winter (r_s = 0.63).

Soil pH relationship altered noticeably after winter. In the flower field, the correlation between pH and nitrate shifted from r_s =-0.82 to r_s =0.66. For oilseed radish, this relationship weakened from r_s = -0.58 to r_s = -0.112. Additionally, the variable that most strongly affected pH post-winter was ACE, shifting from r_s = -0.13 to r_s = -0.60, with a similar trend observed from active carbon in the oilseed radish treatment.

Phase 2:

Before the treatment in the flower field, FDA and Active carbon exhibited a strong, positive correlation (r_s =0.83). However, after spraying, this relationship decreased to non-correlation (r_s = 0.17), while ploughing remained at approximately the same levels (r_s = 0.66). Furthermore, ploughing of the flower field resulted in a strong correlation among all biological aspects (FDA, ACE, Active carbon, and respiration) (r_s = 0.6 to 0.94). In contrast, oilseed radish didn't show the same trend and resulted in a negative correlation between respiration and Active carbon, FDA, and ACE (r_s = -0.5). Comparing before and after spraying shows that spraying for the flowering field disturbs the microbiology (ACE, FDA, Active carbon, respiration; r_s = -0.64 to 0.43) and that ploughing increases the correlation between them (r_s = 0.6 to 0.94). Oilseed radish shows different results for FDA, ACE, and respiration, where it becomes more correlated (rs = 0.6 to 0.83), but lower for Active carbon and ACE, FDA (r_s = -0.31 and -0.-60). (figure 11).

Nitrate is negatively correlated with the biology analysis after ploughing for the flower field (r_s = -0.26 to -0.58). For spraying it changed little, to not being negative to being more non-correlated (r_s = -0.12 to 0.26) except for FDA (r_s = -0.83). Oilseed radish reacted more to the spraying than ploughing, were the spraying was negatively correlated (r_s = -0.6 to -0.89) except for Active carbon (r_s = 0.6)

4. Discussion

This study was made to see if the flowering field had a different effect than the "business as usual" system in Sweden, which consists of oilseed radish after a cereal crop and on the soil, in terms of biology and chemical properties. The experiment underwent some errors, including the fact that the oilseed radish didn't reach its full potential in terms of growth, and some of them still had new growth after winter. Additionally, Red clover hadn't fully died off after glyphosate treatment for the last sampling date.

4.1 Phase 1

Microbial effect on soil

Both the flowering field and the oilseed radish showed positive changes across most of the biological analyses, indicating improved soil health. However, there were two analyses that showed some differences: POXC and FDA. While there wasn't a significant difference in the FDA between the flowering field and the oilseed radish. A trend was observed between the seed mixes, where the flowering field had a higher microbial activity change after the winter ($F_{1.17}$=3.9; P=0.062) and that it also had a higher observed value, post-treatment (Figure 1: Appendix 6; Table 6). Tyler (2020) also observed that the microbial activity increased over time, and fields with a crop had higher FDA than those without. It is possible that the FDA would have been higher if the test were taken later. As higher temperatures promote microbial activity Green et al. (2006). and increase the growth of the flowering field. However, the oilseed radish didn't reach its full potential before winter, and didn't die completely after winter, which could have influenced the amount of FDA in the soil (Appendix 6; Table 6).

While most microbial analyses showed a positive change after winter, POXC showed a negative change for both seed mixes. Before winter, a positive correlation between active carbon and FDA was observed in the flowering field, whereas no correlation was found after winter. Interestingly, the opposite trend occurred for oilseed radish, where the correlation became stronger after winter (Figure 10). Bongiorno et al. (2019) noted that higher organic matter increased active carbon, and since FDA is a measure of microbial activity based on enzymatic production from living cells (Lundgren 1981), and carbon is a source of food. The variation in active carbon could explain the contrasting correlation patterns between the flowering field and the oilseed radish. Since most of the oilseed radish died during the winter, releasing more carbon into the soil, and the

temperatures are too low for the flowering field to start growing and releasing exudates into the soil.

Roper et al. (2019) observed that ACE and yield were correlated by r^2=0.45, which is one aspect of soil health. This study shows that ACE changes positively after winter and, according to Roper et al. (2019), may increase the upcoming yield. However, since there was no difference in the change of the seed mixes, the flowering field isn't the "better" choice in this matter. An explanation for why ACE is positively correlated to yield can be that it is positively correlated to ammonium for both mixes. Mostly for the flowering field, going from r^2=-0.43 before winter to r^2=0.63 after winter. While for oilseed radish, ACE was correlated with both ammonium (r^2=0.52 to 0.60) and nitrate (r^2=0.48 to 0.51). Thus, insinuating that ACE and nitrogen affect each other, and that it is a better tool to predict nitrogen in oilseed radish than in flowering fields. Since they correlate with each other.

Nitrogen dynamics in the soil

Nitrate levels differed significantly between the seed mixes, with the flowering field showing a greater change than oilseed radish. Ammonium levels did not differ significantly between the two seed mixes after the winter, but both showed a decrease. Overall, the flower mix maintained higher observed nitrate levels and a similar ammonium level to the oilseed radish, resulting in a greater amount of plant-available nitrogen in the soil for the sequence crop after the winter. Most probably since the flowering field includes legumes, which are proven to increase soil nitrogen through BNF (Vance 1998; Ross et al. 2009; Riesinger & Herzon 2010; Parr et al. 2011; Jani et al. 2016; Moore et al. 2020; Yang et al. 2020; MacMillan et al. 2022; Weinert et al. 2023; Andersen et al. 2025).

An interesting dynamic was observed in the correlation between nitrate and ammonium before and after winter. For the flower mix, these nutrients were strongly negatively correlated before winter but showed no correlation after winter (r^2=-0.83 to -0.02). The oilseed radish exhibited the opposite trend, with no correlation before winter and a high correlation after winter (r^2= 0.17 to 0.70). This can be because the flowering field has much more ammonium than nitrate before winter, and after winter, plants are still growing, balancing the nutrients in the soil. While the oilseed radish takes up much of the nitrate before winter (Kristensen & Thorup-Kristensen 2004), it then releases it during the winter. While, there are there are no plants to take up the nitrogen, it becomes free in the soil (Elhakeem et al. 2023).

Based on the results, the flower fields improve nitrogen levels after winter. However, whether this is due to the winter effect or the microbial effect is hard to determine. Since the study's results show that microbial analysis is not well correlated with nitrate and ammonium after winter. Even though oilseed radish

soil has a slightly higher correlation between the nitrogen and microbial analysis than the flowering field. (Figure 10).

4.2 Phase 2

Microbial effect on the soil

Both the flowering field and the oilseed radish showed positive changes across most of the biological analyses in both treatments, indicating improved soil health. However, there were some microbial analyses showing differences between the treatments and mixtures. The microbial activity for the oilseed radish had an increase, while the flower field had a negative change after the treatments (Figure 1: Appendix 6; Table 7). This can be due to the fact that, some microorganisms can degrade glyphosate and use it as a food source (Shinabarger & Braymer 1986; Dick & Quinn 1995; Singh & Walker 2006; Ermakova et al. 2017; Firdous et al. 2020). Since the flower field was proven to have a reduction in the FDA can be due to the fact that the plants wasn't fully decomposed yet and was still green (Elbl et al. 2019). This could affect the microorganism's decomposition. To see the effect of microbial activity, one has to wait for a longer time as these processes take time to start the decomposition (Giweta 2020). This is especially true for the flowering field, which has a higher aboveground biomass that demands extended microbial processing time since there is a higher biomass aboveground for the flowering field (Xiao et al. 2017). It would also be interesting to see diversity of the microorganism since Chiba et al. (2021) observed that decomposition was correlated positively with soil bacteria diversity. And since flowering field has more species in the mix, it would possibly increase the biodiversity in the soil.

Furthermore, POXC had the opposite reaction to the FDA; according to the correlation. Flowering field had one of the highest correlations between active carbon and FDA before the treatments but lost it after spraying (Figure 11). However, the flowering field that was ploughed only lost some correlation. This can then be interpreted that active carbon does not correspond to microbial activity if sprayed and somewhat to ploughed. Which can be due to whether there was much vegetation or not. Which is not what Jensen et al. (2019) found, where POXC is higher when there are vegetation. Where this study results shows the opposite (Appendix 6; Table 10). However, since the sample date for before the treatment was at the beginning of March (Appendix 6; Table 1), the temperature wasn't high enough for optimum growth, thus reducing the active carbon release from the root exudates into the soil (Gong et al. 2022). This can also be one explanation why the oilseed radish wasn't affected as much since there wasn't that much growing (Nadjire et al. 2025) (Appendix 6; Table 10).

Nitrogen dynamics in the soil

When comparing the nitrogen level for the flower field or the oilseed radish there were no significant change between them from either seed mix or treatment (Figures 5 and 6). However, looking at the observed value for nitrate, it showed that even though the change wasn't significant for the seed mixes, oilseed radish had a higher amount of nitrate than the flowering field (Appendix 6; Table 12). Possibly, since the flowering field has much of the nitrate still in the organic bound form, as roots have a high C/N-ratio, resulting in a slower decomposition rate than for the oilseed radish, which has been decomposing since the winter. Müller & Berg (1988) observed that it takes approximately 30 days for 70% of the red clover root biomass to decompose. Yang et al. (2020) observed that shoots and roots decompose at different rates, with shoots decomposing faster than roots, possibly due to differences in C/N-ratios. Suggesting that the nitrogen is immobilized in decomposition processes (Vigil & Kissel 1991) and for this experiment, it would probably have higher nitrate levels if tests had been taken one or two weeks after the treatments (Adhikari et al. 2024).

Treatment effect on microbial aspect

The most effect from the different treatments was for FDA and POXC, while the other analysis wasn't affected as much. POXC had significantly greater change in the flower mix ploughed than in the sprayed; however, it was not significantly different from the other treatments of oilseed radish.

Although Bongiorno et al. (2019), observed a higher POXC in non-ploughed fields. Which is the opposite of the result in this study, where POXC was significantly higher in ploughed than spraying in the flower fields, can be explain partly to the timing of the sampling date. The red clover wasn't completely dead when taking the samples after treatment, thus retaining much of the carbon within itself. Combined with the high C:N-ratio for both the English ryegrass and red clover roots, it was too early to see the release and decomposition of the carbon in the sprayed (Lak et al. 2020; Yang et al. 2020). Conversely, incorporation through ploughing had a more immediate effect because the biomass was directly buried in the soil (Jahanzad et al. 2016). While intensive tillage and soil disturbance are well known to deplete organic carbon stocks over time (Bot & Benites 2005), the immediate, short-term effect of incorporating fresh organic matter is an increase in localized POXC pools (Awale et al. 2017).

Regarding the FDA, the sprayed treatment exhibited significantly greater change than both the flower fields treatment, suggesting an increased decomposition rate. This can be explained by the fact that glyphosate can be a food source for certain microorganisms (Shinabarger & Braymer 1986; Dick & Quinn 1995; Singh & Walker 2006; Ermakova et al. 2017; Firdous et al. 2020).

The oilseed radish plots also had lower growth, thus died faster than the flower fields, where the English ryegrass and red clover had a visually higher biomass.

Prior to treatment, FDA and POXC showed a very high correlation. After treatment, only the ploughed showed some correlation, while the sprayed showed none. It was proven that the ploughed showed high correlation between most of the biological parameters (ACE, FDA, respiration, Active carbon ranking from r_s=0.60 to 0.94). So even if all of the microbial analysis didn't show significance, the ploughed field for the flowering field is more closely related to the microbiology than the sprayed flowering field.

Treatment effect on nitrogen dynamics

The effect seen from the winter was lost to the treatments, as there were no significance between the treatments. However, oilseed radish exhibits a higher amount of nitrate, regardless of the treatment. While, ploughing exhibited some positive correlation (r^2=0.41 and 0.30) between nitrate and ammonium for both the seed mixes (Figure 11). This indicates that oilseed radish residue had already undergone mineralization in the soil since the winter. Conversely, the flower mix that was still alive seems to take a longer time to see a higher amount of nitrogen being released in the soil (Yang et al. 2020). Theoretically, when looking at the amount of nitrogen, the farmer could save approximately 14 kg ha^{-1} for the flowering field and 20 kg ha^{-1} for the oilseed radish (Table 12).

To fully evaluate these dynamics, it would have been valuable to analyze the nitrate level deeper in the soil profile alongside total soil nitrogen. This would help determine whether the oilseed radish treatments led to nitrogen leaching, since nitrogen leaching in sandy soils is high in the early season (Stenberg et al. 1999; Kristensen & Thorup-Kristensen 2004). To get the full picture of nitrogen dynamics, total nitrogen levels would have been a useful measure. Since the flower field has legumes, nitrogen could have been in an organic form. Thus not showing in the Spurway analysis. Lastly, assess the C/N ratio to determine whether it is high in the flowering field, thereby promoting decomposition and leading to immobilization (Bot & Benites 2005).

5. Conclusion

Microbial activity: Soil microbial responses were parameter-specific rather than uniform. Overall, differences between seed mixes were limited. The two-way ANOVA revealed that the FDA differed significantly between the mixes. The post hoc Tukey HSD test confirmed that only the oilseed radish sprayed was significantly different from the flowering mix-treated (not sharing a letter). For POXC, a significant treatment effect was observed in the two-way ANOVA. The Tukey HSD test proved that the ploughed flower mix was significantly higher than the sprayed flower field.

Nitrogen dynamics: The winter-phase effect had a significant impact on nitrate levels between mixes. The Tukey HSD test proved that the flower fields had significantly greater change in levels of nitrate than the oilseed radish. However, this effect was lost by the spring, as no significance was observed between the treatment and the seed mix. Nevertheless, the oilseed radish accumulated more nitrate overall, both before and after treatments, than in the flower fields. Even though the total amount was low, approximately 14 kg nitrate ha^{-1} for the flower field and 20 kg nitrate ha^{-1} for the oilseed radish. This indicates that the winter effect was the main driver of nitrogen dynamics, rather than the spring treatment.

Overall trends: The majority of the biological analyses showed a positive net change following both the winter period and the spring treatments. The notable exceptions to this trend were the FDA and POXC. POXC showed a net negative change after winter for both seed mixes, whereas FDA showed a net negative change for the flower mix following the spring treatments.

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Popular science summary

Flowering Fields: A Feast for the Eyes or a Cure for the Soil?

In previous years, the edges of flowers have developed into entire fields of flowering plants, grabbing the attention of the public eye. This development is based on funding to increase biodiversity and our pollinators. However, can this subsidy be a piece of the puzzle that solves two of the most critical aspects in agriculture, rising fertilizer prices and a changing climate? Not for the flowers but from the roots of the flowering plants, discovering a whole new aspect of seeing the flowers.

By Filip Åkerblom

Modern farming faces a double-edged sword: an increasingly unpredictable climate and fluctuating fertilizer costs. All due to the sensitive world happenings such as the Hormuz closure and the war in Ukraine. Could the flowering field be used as a synergetic effect to both increase the biodiversity and lower the need for nitrogen and increase the resilience to the climate?

To investigate this, a field experiment was conducted in Scania, southern Sweden. Where a flower mix of eight species (*Red, Crimson, Persian, and Yellow clovers alongside oilseed radish, ryegrass, buckwheat, and Lacy Phacelia*), was tested against a traditional cover-cropping system (*spring barley* followed by *oilseed radish*). Different analyses were conducted to compare nutrient and biological parameters over the winter and under different soil management in the spring, which included chemical herbicide spraying or mechanical plowing.

The Winter Nitrogen Boost

Nitrogen fertilizers are highly important in the agricultural and horticultural system, in which they are a limiting factor to yield. Winter was shown to significantly increase nitrate levels in the flowering mix compared to traditional oilseed radish fields. This may be because nitrogen-fixing legumes in the flower mix pull free nitrogen from the air in the soil.

However, nitrogen is very in the form of nitrate is very labile in the soil. This was concluded since the increase of nitrate in the flowering mix was lost throughout the spring. Oilseed radish proved better at locking down and maintaining high nitrate levels later into the season, regardless of whether the field was plowed or sprayed. This highlights a classic agricultural hurdle: creating nutrients in the soil is only half the battle; timing their release so that the subsequent crop can absorb them before they leach away is the real challenge.

Feeding the Microbes: A Tailored Diet

Soil health is a widely used term by both professionals and hobbyists; however, the EU and the USA have defined it as comprising three parameters: chemical, physical, and biological. Soil can be seen as “dead” but is very much alive and has a highly complex system, so to analyze the soil, all three parameters have to be included, since they affect one another.

When the fields were terminated in the spring, the soil microbes responded strictly to their treatment. Microbial activity increased sharply in the oilseed radish plots that were chemically sprayed, likely because certain microorganisms use these compounds as a temporary food source. On the flip side, active carbon received a major boost in the flowering fields that were mechanically ploughed. This demonstrates that soil biology is highly sensitive and responds specifically to a farmer's management choices and the "diet" provided by different seed mixes.

A Colorful Piece of the Sustainable Puzzle

Is the flowering field the silver bullet for agriculture and horticulture in the upcoming challenges? Not quite, as for all systems, there are good and bad aspects where one has to weigh them against each other. Since the soil is a highly complex system, it is hard to have a “golden rule,” and every field needs to tailor to its needs. As this study shows, it is difficult to conclude whether the flowering field is the best alternative, and evaluating it requires long-term research to determine exactly how it affects the yield of the subsequent crop. However, I think that flowering field will be seen more and more in the landscape because it has the potential to build resilience and reduce synthetic fertilizers.

Acknowledgements

I would like to thank many people for helping me with this paper, both in terms of the writing and also for the support.

Thanks to HIR Skåne, especially Oskar Hansson, for the help and planning of this work; it has been interesting to gain insight into the work of an advisor.

Thank you, Lisa-Marie, for supporting me when the workload was heavy. I don't think I would have been able to finish the paper otherwise.

Thanks to my family and friends, Johan, Peter, Rebecca, Arielle, Inger, Sten-Åke and Markus for being supportive and helpful when I was stuck in work and for putting me in my place. You remind me to stay humble and never to think that I am better than others, even though I am a poet now…..

Lastly, I would like to thank my supervisor, Anna-Karin Rosberg, for being helpful and understanding of my work and for providing me with the right tools to complete this work. I recommend other students to have you as a supervisor in the future.

Rajinar di så …. vi
Rajinar di ente så…. Vi endå
Skål bönner!

Appendix 1

Table 2: Overview of the management of the experiment.

Month / Year	Date	Spring barley (SB) + oilseed radish (OR)	Flowering field (FF)
April 2025	09	Soil sample, no. 1	
	17	Sown SB (variety Lexy 210kg) + 100 kg N ha^{-1}	N/A
May 2025	12	N/A	Sown FF + 40 kg N ha^{-1}
July 2025	08	Biomass sample, no. 1	
	07	N/A	Trimming
August 2025	06	Harvesting SB	Trimming
	XX	Harrowing SB plots	N/A
	13	Sown OR	N/A
	14	Irrigation, 15 mm	
	15	Irrigation, 15 mm	
	16	Irrigation, 15 mm	
November 2025	07	Biomass sample, no. 2	
December 2025	09	Soil sample, no. 2	
March 2026	12	Soil sample, no. 3	
April 2026	27	Soil sample, no. 4 (Before glyphosate and ploughed)	
	29	Irrigation 20mm	
	30	Glyphosate treatment (6L)	
May 2026	04	Plowed and harrowed treatment	
May 2026	11	Soil sample, no. 5 (After glyphosate and ploughed)	

Appendix 2

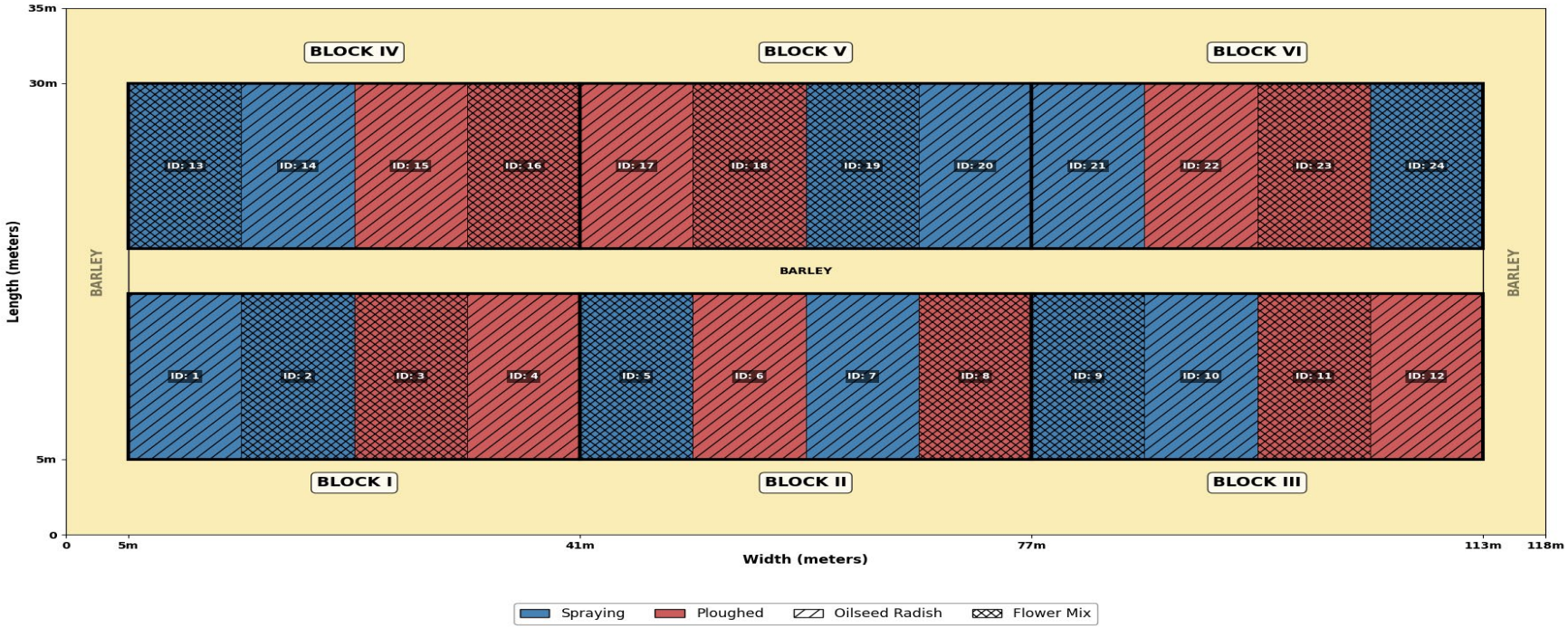


Figure 9: Schematic field layout of the experimental design divided into six blocks (Blocks I–VI), surrounded by a barley buffer zone. Each individual plot is labelled with a unique identification number (ID 1–24). The figure illustrates the distribution of the two treatment methods, Spraying (blue plots and Ploughed (red plots), combined with the two plants mixes, Oilseed Radish (diagonal hatching) and Flower Mix (cross hatching).

Appendix 3

Table 3:Flower mix species, with cost (SEK/kg) and amount (kg/ha), for the mix.

Species	Kg/ha	Variety	SEK/kg (at minimum 100 kg)	Cost in the mix SEK:-
Fagopyrum esculentum (Common buckwheat):	10	N/A	23	230
Phacelia tanacetifolia (Lacy Phacelia or Tansy Phacelia):	3	N/A	54	162
Raphanus sativus L. var. oleiformis Pers (oilseed radish):	3	Cordoba	55	165
Crimson clover (Trifolium incarnatum)	5	N/A	42	210
Trifolium resupinatum (Persian clover or reversed clover):	3	N/A	71	213
Trifolium pratense (Red clover)	5	N/A	124	620
Lolium perenne (English rye grass):	4	N/A	36	144
Melilotus officinalis (sweet clover or melilot):	3	N/A	59	177
Total kg/ha: Total cost of the mix:	36			1921 kr

Appendix 4

Table 4. Support areas for different funding areas based on where the field is located, 2025 (Jordbruksverket 2026).

Support Area	Payment/ha (€)	Range/ha (€)
Compensation support area (1–12)	235	212–282
Outside the compensation support area, within the southern plains of Götaland (13a)	663	596–729
Outside the compensation support area, outside the southern plains of Götaland (13b)	361	325–434

Below are the general demands for the flowering field and field edges: *(If you are interested in more details of the rules, please visit their website. See Jordbruksverket.)*

- Before applying for this subsidy, you must follow the general conditions. See Jordbruksverket.
- The field is not allowed to be in production from the 1 of Jan to the end of the break point.
- No pesticides are allowed to be used on the designated area from 1 January until the break point.
- Three of the approved pollen- or nectar-rich herb has to be sown.
- The herbs have to be sown so that they are established and visually seen before 15 of July and that they flower before the field is allowed to be in production again.
- Do not till the plot from when the herbs are established until the breaking points.
- Do not trim your flowering field and field edges during the period from 1 April to 30 June. *(You are not allowed to trim if the field is in full flower or if it excludes the late flowering species.)*
- Not allowed to disturb the flowering field and field edges before the breaking point. The exact date depends on where your land is located.
  - For fields in Blekinge, Skåne, or Halland counties: not earlier than 20 October.
  - For fields in all other regions: not earlier than 10 October
- The flowering field and field edges cannot be used for production in the following year.
- Every area must be at least 0,1 ha.

Appendix 5

Table 5: Programs used in R, with version and uses of separate programs.

Package	Version	Primary function
R (Core)	4.3.1	Base statistical environment
readxl	1.4.5	Importing data from Excel spreadsheets
tidyverse	2.0.0	Collection of packages for data science and formatting
emmeans	2.0.3	Calculation of Estimated Marginal Means
multcomp	1.4.30	Simultaneous inference and post-hoc tests
multcompView	0.1.11	Visualizing significant differences (letter displays)
ggplot2	4.0.3	Creating high-quality data visualizations
ggfortify	0.4.19	Visualizing statistical model diagnostics
ggcorrplot	0.1.4.1	Visualizing correlation matrices
dplyr	1.1.4	Data manipulation and transformation
car	3.1.3	Conducting Levene's test for homogeneity of variance and advanced ANOVA modeling
lubridate	1.9.4	Handling, parsing, and manipulating date and time variables
patchwork	1.3.2	Combining and arranging multiple ggplot2 plots into customized layouts

Appendix 6

Table 6 :Observed values of FDA Phase 1 (mg kg^{-1}) before (Pre) and after (Post) winter, and the calculated difference (Δ) during Phase 1 (Winter effect). Pre- and post-treatment values represent observed means.

Mix	Pre-Treatment	Post-Treatment	Difference (Δ)	Standard error (SE)
Flower Mix	0.199	0.295	0.095	0.0157
Oilseed Radish	0.203	0.254	0.051	0.0157

Table 7: Observed values of FDA Phase 2 (mg kg-1) before (Pre) and after (Post) termination treatments, and the calculated difference (Δ) during Phase 2 (Treatment effect). Pre- and post-treatment values represent observed means.

Mix	Treatment	Pre-Treatment	Post-Treatment	Difference (Δ)	Standard error (SE)
Oilseed Radish	Sprayed	0.242	0.281	0.039	0.0148
Oilseed Radish	Ploughed	0.234	0.241	0.007	0.0148
Flower Mix	Ploughed	0.251	0.204	-0.046	0.0148
Flower Mix	Sprayed	0.259	0.212	-0.046	0.0148

Table 8: Observed values of Respiration Phase 2 (CO2-C kg^{-1} d^{-1}) before (Pre) and after (Post) termination treatments, and the calculated difference (Δ) during Phase 2 (Treatment effect). Pre- and post-treatment values represent observed means.

Mix	Treatment	Pre-Treatment	Post-Treatment	Difference (Δ)	Standard error (SE)
Oilseed Radish	Sprayed	14.31	14.5	0.25	4.23
Flower Mix	Sprayed	17.4	19.82	2.42	4.23
Flower Mix	Ploughed	18.12	13.71	-4.41	4.23
Oilseed Radish	Ploughed	8.78	9.24	0.46	4.23

Table 9: Observed values of Active Carbon Phase 1 (mg g^{-1}) before (Pre) and after (Post) winter, and the calculated difference (Δ) during Phase 1 (Winter effect). Pre- and post-treatment values represent observed means.

Mix	Pre-Treatment	Post-Treatment	Difference (Δ)	Standard error (SE)
Oilseed radish	577.15	503.53	-73.61	27.4
Flower mix	571.80	522.23	-49.57	27.4

Table 10: Observed values of Active Carbon Phase 2 (mg g^{-1}) before (Pre) and after (Post) termination treatments, and the calculated difference (Δ) during Phase 2 (Treatment effect). Pre- and post-treatment values represent observed means.

Mix	Treatment	Pre-Treatment	Post-Treatment	Difference (Δ)	Standard error (SE)
Flower_mix	Ploughed	519.61	639.63	120	27.6
Oilseed_radish	Spraying	509.56	534.68	25.12	27.6
Oilseed_radish	Ploughed	524.63	560.36	35.72	27.6
Flower_mix	Spraying	515.14	509.0079	-6.140573	27.6

Table 11: Observed values of Nitrate Phase 1 (kg N ha^{-1}) before (Pre) and after (Post) termination treatments, and the calculated difference (Δ) during Phase 2 (Treatment effect). Pre- and post-treatment values represent observed means.

Mix	Pre-Treatment	Post-Treatment	Difference (Δ)	Standard error (SE)
Flower mix	5.67	29.37	23.7	1.98
Oilseed_radish	5.65	21.27	15.6	1.98

Table 12: Observed values of Nitrate Phase 2 (kg N ha^{-1}) before (Pre) and after (Post) termination treatments, and the calculated difference (Δ) during Phase 2 (Treatment effect). Pre- and post-treatment values represent observed means.

Mix	Treatment	Pre-Treatment	Post-Treatment	Difference (Δ)	Standard error (SE)
Flower_mix	Ploughed	15.39	14.56	-0.83	1.71
Oilseed_radish	Ploughed	22.49	22.57	0.06	1.71
Flower mix	Spraying	15.64	13.73	-1.97	1.71
Oilseed_radish	Spraying	21.77	19.92	-1.84	1.71

Appendix 7



Figure 10: Spearman rank correlation matrix of soil properties across two different sampling timesteps (Before_winter and After_winter) for Flower_mix (top row) and Oilseed_radish (bottom row). The heatmaps illustrate the strength and direction of monotonic relationships between soil parameters, with the correlation coefficient (ρ) displayed inside each tile. The color scale ranges from dark orange, indicating a strong negative correlation (-1.0), to dark blue, indicating a strong positive correlation (+1.0). White tiles represent no linear or monotonic correlation (≈ 0.0).

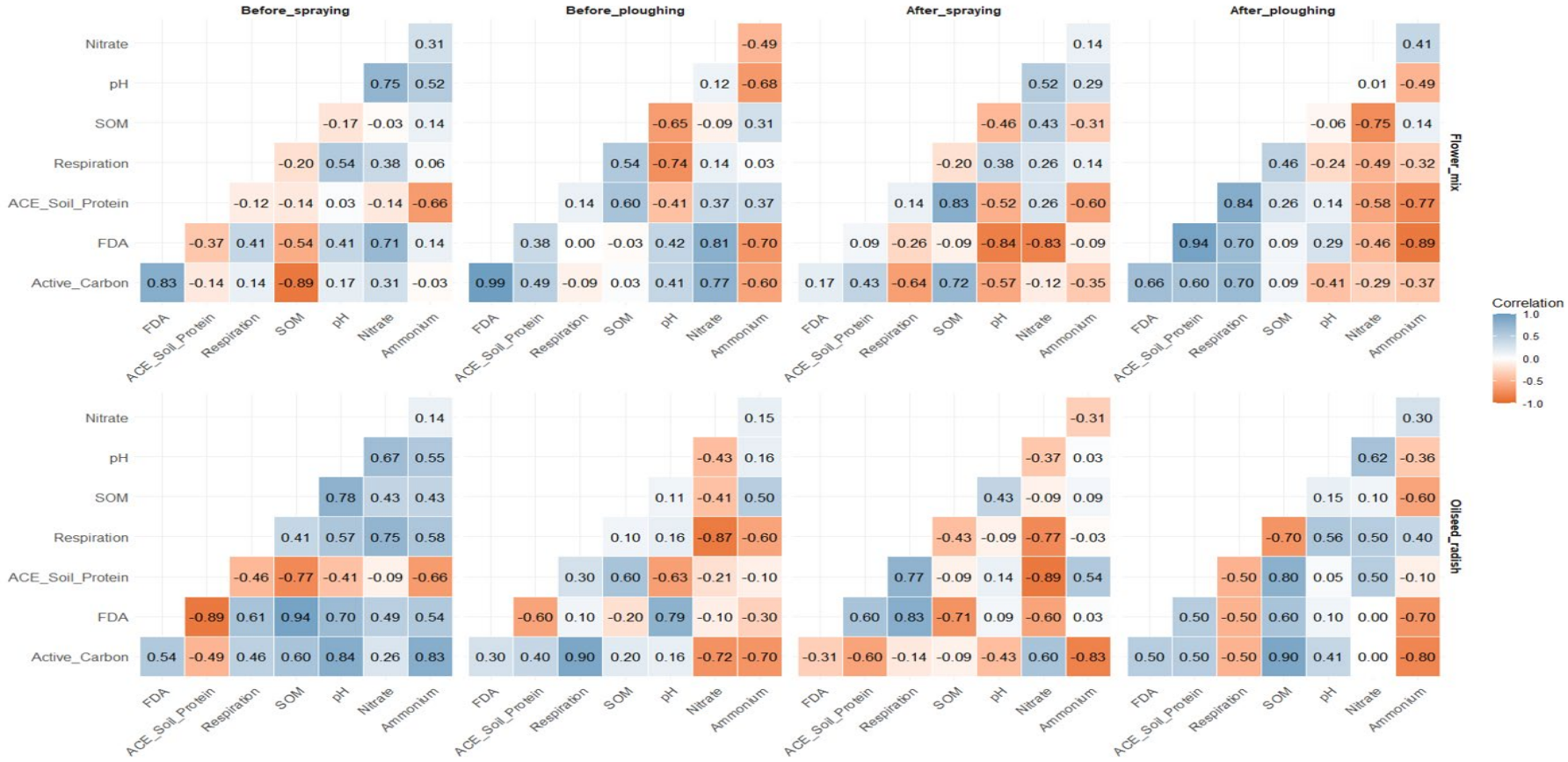


Figure 11: Spearman rank correlation matrix of soil properties across four different sampling timesteps (Before_treatment and After_treatment) for Flower_mix (top row) and Oilseed_radish (bottom row). The heatmaps illustrate the strength and direction of monotonic relationships between soil parameters, with the correlation coefficient (ρ) displayed inside each tile. The color scale ranges from dark orange, indicating a strong negative correlation (-1.0), to dark blue, indicating a strong positive correlation (+1.0). White tiles represent no linear or monotonic correlation (≈ 0.0).

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