# Underlying mechanisms behind nitrous oxide emissions in oilseed radish, *Raphanus sativus var. oleiformis*, and *Phacelia tanacetifolia*

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Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Plant Breeding
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# Underlying mechanisms behind nitrous oxide emissions in *Raphanus sativus var. oleiformis*, and *Phacelia tanacetifolia*

*Underliggande mekanismer bakom lustgasutsläpp i oljerättika,* Raphanus sativus var. oleiformis*, och honungsört,* Phacelia tanacetifolia

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## Abstract

Greenhouse gases are a driving force of climate change and the annual greenhouse gas emissions were higher between 2010-2019 than any other time in human history (Skea et. al. 2022). Cover crops are used to mitigate the effect of climate change, but recent studies indicate that the use of the cover crop oilseed radish results in significantly larger emissions of nitrous oxide ($N_2O$) compared to other cover crops (Dörsch et. al. 2022; Müller Júnior et. al. 2019; Olofsson and Ernfors 2022; Thomas et. al. 2017). Results from Olofsson and Ernfors (2022) study showed that *Raphanus sativus var oleiformis* (OR) causes significantly higher emission of $N_2O$ compared to *Phacelia tanacetifolia* (PH) even though the quality and quantity were similar. The hypothesis in this study was that the glucosinolates in OR provides a carbon source for denitrifying bacteria, thus causing a significantly higher $N_2O$ emission compared to PH. The hypothesis was tested in a laboratory setting during 32 days of gas measurement with plant material of OR and PH incubated with and without added glucose. Surprisingly, aboveground plant material of PH showed highest emissions throughout the study. The result of this study could not confirm the hypothesis since added glucose did not affect the $N_2O$ emission. In further studies, it is recommended to increase the number of replicates and optimize the methodology to be able to draw any conclusions.



*Keywords:* nitrous oxide, glucosinolates, greenhouse gases, oilseed radish, *Phacelia tanacetifolia*, cover crops, labile C

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# Abbreviations

| | |
|---|---|
| A | Aboveground |
| AB | Aboveground plus belowground |
| ABG+ | Aboveground, belowground plus glucose |
| C | Carbon |
| CC | Cover crop |
| $CH_4$ | Methane |
| $CO_2$ | Carbon dioxide |
| Dw | Dry weight |
| GHG | Greenhouse gases |
| GSLs | Glucosinolates |
| GWP100 | Global warming potential on a 100-year scale |
| Ha | Hectare |
| ITCs | Isothiocyanates |
| N | Nitrogen |
| $N_2$ | Nitrogen gas |
| $N_2O$ | Nitrous oxide |
| $NH_3$ | Ammonia |
| $NH_{4+}$ | Ammonium |
| $NO_{2-}$ | Nitrite |
| $NO_{3-}$ | Nitrate |
| $O_2$ | Oxygen |
| OR | Oilseed rape, *Raphanus sativus var. oleiformis* |
| PH | *Phacelia tanacetifolia* |
| SE | Standard error |
| SITES | Swedish Infrastructure for Ecosystem Science |
| WFPS | Water-filled pore space |

# 1. Introduction

Greenhouse gases (GHGs) are a driving force of climate change and the annual GHGs emissions were higher between 2010-2019 than any other time in human history (Skea et. al. 2022). Agriculture is an important driver of climate change, both through on-farm emissions linked to production and land use change due to agricultural expansion (OECD 2022). Agriculture, forestry, and other land use represents 22% of the global anthropogenic GHG emissions. Half of the emissions is from on-farm emissions of methane ($CH_4$) and nitrous oxide ($N_2O$), and the other half is carbon dioxide ($CO_2$) emissions from land use, land use change and forestry (OECD 2022). $N_2O$ is responsible for approximately 6% of the enhanced greenhouse effect, which is when additional radiative forcing resulting from increased concentrations of GHG induced by anthropogenic actions (Ussiri and Lal 2013; WMO 2022). Since pre-industrial times, the tropospheric abundance of $N_2O$ was 270 ppb but 332 ppb in 2019 – an increase by 23% (Canadell et. al. 2021).

$N_2O$ is a long-lived atmospheric trace gas with a lifetime average of 116 years (Canadell et. al. 2021). Lately, it has received great attention because it is also a crucial factor in depleting the ozone layer (Ravishankara et. al. 2005). Compared to the other GHG $CO_2$, the heat trapping effect is 273 times more powerful (Nabuur et. al. 2022). One of the main sources of human induced $N_2O$ emission is agricultural soils (Mitchell et. al. 2013). About 60-80% of global $N_2O$ emissions derive from agriculture (Ussiri and Lal 2013). Between the period 1990 and 2019, agricultural emissions of $N_2O$ increased by 9% (EPA 2021).

Recently, an interest in incorporating cover crops into the crop rotation has increased. Cover crops (CCs) have been proposed to mitigate climate change by a natural process of removing $CO_2$ from the atmosphere by carbon (C) sequestration (Aronsson et. al. 2012). CCs are grown for the protection and enrichment of soils yet not cultivated for its harvest of biomass. There are many environmental benefits with CCs. To mention only a few, they can reduce nutrients leaching into aquatic ecosystems, increase C sequestration and benefit pollinators in the surroundings (Aronsson et. al. 2012). However, there is a tradeoff between the negative and positive outcomes of CCs. Studies have shown that cover crops can increase

emission of $N_2O$ (Mitchell et. al. 2013; Müller et. al. 2003; Olofsson and Ernfors 2022).

In the coming decades, $N_2O$ emission is expected to continue to increase because of the growing demand for food and energy (Tian et. al. 2020). But which setting or situation that drives the $N_2O$ fluxes is still investigated. To reduce emissions, a better understanding of the mechanisms of the sources and sinks behind $N_2O$ emission when incorporation CCs in the crop rotation needs to be assessed. Recent studies indicate that the use of the CC oilseed radish results in significantly larger emissions of $N_2O$ compared to other CCs (Dörsch et. al. 2022; Müller Júnior et. al. 2019; Olofsson and Ernfors 2022; Thomas et. al. 2017). Results from Olofsson and Ernfors (2022) study showed that oilseed rape, *Raphanus sativus var oleiformis* (OR) causes significantly higher emission of $N_2O$ compared to *Phacelia tanacetifolia* (PH) even though the quality and quantity were similar.

The net value of benefits needs to be considered growing OR, especially as a CC. However, there is a knowledge gap of which underlying processes cause the emission of $N_2O$ in OR. The experiment presented below compares the $N_2O$ emission between *Raphanus sativus var. oleiformis* and *Phacelia tanacetifolia*. The comparison is made between aboveground and belowground plant residues with and without added glucose to examine what mechanism behind cause $N_2O$ emission during a freeze and thaw treatment.

# 2. Background

## 2.1 Soil Emission of $N_2O$

The nitrogen cycle is in a constant change of chemical forms. The processes behind $N_2O$ formation are complex and affected by several different factors. Nitrification and denitrification's contribution to form $N_2O$ depends on environmental factors (Wang et. al. 2005). Nitrification oxidises ammonium ($NH_4^+$) or ammonia ($NH_3$) to nitrate ($NO_3^-$) via nitrite ($NO_2^-$) under aerobic conditions (Ussiri and Lal 2013). During denitrification, $NO_3^-$ is reduced to $N_2O$ during organic carbon oxidation under anaerobic conditions (Mitchell et. al. 2013). In most cases, during certain conditions $N_2O$ is reduced into $N_2$ and complete the N cycle.

Whether the denitrification product is $N_2O$ or $N_2$ depends on environmental factors. It is a complex process and some of the underlying mechanisms are still unknown. According to Ussiri and Lal (2013) the ratio between $N_2O/N_2$ depends on the oxygen supply, water-filled pore space (WFPS), decomposable organic carbon, N substrate supply, temperature, pH, and salinity. The processes forming $N_2O$ and $N_2$ can occur separately or simultaneously depending on the soil air and availability of substrate.

For denitrification, the optimum WFPS of 70-80% (Butterbach-Bahl et. al. 2013) and with a pH within the range of 7.0-7.5 (Saleh-Lakha et. al. 2009). $N_2O$ emissions are sensitive to rising temperatures, not the temperature per se but the increased microbial activity that increases soil respiration and creates anaerobic hotspots (Butterbach-Bahl et. al. 2013).

A high clay content has been found to lower $N_2O$ emissions (Abalos et. al. 2022). This can be since a high clay content decreases soil aeration and oxygen ($O_2$) availability, thus decreasing residue releasing N from decomposition which promotes $N_2O$ reduction to $N_2$ via a complete denitrification. This goes in line with

Ussiri and Lal (2013) meaning that dry soil inhibits nitrification and waterlogged soil increases the denitrification rate and formation of $N_2$ - thus closing the nitrogen cycle.

Microbes are responsible for a large part of the global production of $N_2O$ and denitrifiers in soil stand for 5% of the soil microbial community (Ussiri and Lal 2013). Microbial transformations in nitrification and denitrification are responsible for 70% of the annual $N_2O$ production in soils. Autotrophic nitrification and heterotrophic denitrification occur in terrestrial environments. However, in cultivated soil denitrification is the most dominant precursor of $N_2O$ production (Mitchell et. al. 2013).

## 2.2 Nitrogen and Carbon Availability

To which extent nitrogen or carbon availability in soils limits or enhances $N_2O$ emission is still largely unknown. According to Abalos et. al. (2022) the most common biochemical property used to predict the effect of crop residues on $N_2O$ emissions is the C/N ratio. Crop residues with a lower C/N ratio than 20-30 are expected to cause N mineralization due to their high N concentration. However, crop residues with a higher C/N ratio than 3 have been found to result in N immobilization. Immobilization of soil N may decrease $N_2O$ emissions since there is reduced availability of ammonium and nitrate for the processes of nitrification and denitrification.

Previously mentioned, the dominant process for $N_2O$ emission in soil is denitrification (Müller et. al. 2003). High emissions of $N_2O$ are correlated with high concentrations of $NO_3^-$ and low concentrations of $NH_4^+$ which indicates denitrification is dominating. Added N fertilizer and $NO_3^-$ often increases $N_2O$ emissions (Mitchell et. al. 2013). Worth noting is that mineralizable C might play a more important role in controlling $N_2O$ emissions than $NO_3^-$ availability.

Available organic carbon in soil influences microbial metabolism since it is utilized directly as the main source of energy (Chen et. al. 2020). Application of organic fertiliser with large amounts of labile C increases the denitrification rate (Senbayram et. al. 2012). Applied C does not only provide microbes a source of energy but also creates an anaerobic microsites due to increased demand for $O_2$ (Azam et. al. 2002). The energy source of available C together with the hypoxic environment creates a favorable environment for denitrification. Studies have shown that addition of labile C increased the rate of $N_2O$ emissions (Mitchell et. al. 2013; Mørkved et. al. 2006; Köster et. al. 2011). Newly available organic carbon in

thawed soil stimulated $N_2O$ emission both by increasing available resources for denitrifiers and stimulating soil respiration (Risk et. al. 2013).

Previously it has been shown that during $NO_3^-$ or $NO_2^-$ reduction to $NH_4^+$, $N_2O$ is produced (Zhang et. al. 2019). According to Streminska et. al. (2011), the ratio between C and $NO_3^-$ had a major influence on the products of nitrate ammonification. $N_2O$ production was at maximum under limited C availability and $NO_3^-$ sufficient conditions in chemostat cultures. Under low C to $NO_3^-$ ratio of 5 and 10 to 1, 2.7% and 5% of the $NO_3$-N being reduced to $N_2O$ by *Bacillus* and *Citrobacter*, but approximately 60-70% of the conversion products were $NO_2^-$. The reduction efficiencies were only 0.1% or 0.7% higher with higher C to $NO_3^-$ ratio of 25 and 50 to 1 (Streminska et. al. 2011). Therefore, it is not certain what environmental conditions which nitrate ammonification contributes to $N_2O$ emission from soil.

Added $NO_3^-$ did not increase $N_2O$ emission according to Mitchell et. al. (2013). But Senbayram et. al. (2012) showed that $NO_3^-$ was limiting denitrification in low concentrations. The $N_2O$ emission quickly declined towards zero with less than 20 mg $NO_3^-$-N $kg^{-1}$ dry soil. A study by Thomas et. al. (2017) indicates that $NO_3^-$ levels less than 6 mg $NO_3^-$-N limited $N_2O$ fluxes. Labile C together with $NO_3^-$ may determine greenhouse gas emissions by regulating microbe's metabolism. If it is an additive, antagonistic or synergistic relation between N and C will be discussed.

## 2.3 Freeze and Thaw Cycles

Production of $N_2O$ is characterized by peaks of emissions with high spatial and temporal variability (Ussiri and Lal 2013). Globally, large rates of $N_2O$ fluxes are induced by soil freeze and thaw cycles (Wagner Riddle et. al. 2017). Freeze and thaw cycles are of special interest because microbes are still active around 0°C and this leads to pulses of $N_2O$ production (Butterbach-Bahl et. al. 2013; Wagner Riddle et. al. 2017). Thawing of soil is an important driver of $N_2O$ emissions due to following factors listed:

1. Substrate availability.
2. Changes in the structure and activity of denitrifying enzymes.
3. Physical trapping and release of previously produced $N_2O$.

During thawing of soil, substrate availability changes because of changes of soil aggregates, the release of nitrate ($NO_3^-$) and C in crop residues, and microbial cell lysis (Wagner Riddle et. al. 2017). According to Mørkved et. al. (2006) only 4.4% of the $N_2O$ emission originated from nitrification, confirming denitrification is the

main source of $N_2O$. The study showed that the freeze-thaw cycle inducing release of decomposable organic C was the driving force of $N_2O$ emissions - both by fuelling denitrifiers and depleting oxygen. An estimation is that 25-37% of the total spring thaw $N_2O$ flux at cultivated sites derives from the physical release of trapped $N_2O$ in the 0-10 cm top layer of the soil profile (Risk et. al. 2013). To conclude, there are two mechanisms proposed to lead to $N_2O$ emissions at thaw - physical release of $N_2O$ produced during winter and newly produced $N_2O$ by biological activity or change in soil conditions.

## 2.4 Cover Crops and $N_2O$ Emissions

To mitigate the effects of climate change, producers incorporate cover crops into the crop rotation to cover the soil rather than being harvested. Cover crops can be used for carbon sequestration and therefore the effect cover crops have on greenhouse gases must be considered. Studies regarding whether cover crops decrease or increase emissions of $N_2O$ is contradictory. Studies have shown that cover crops can either reduce $N_2O$ emissions (Reicks et. al. 2017), as well as not significantly affect the $N_2O$ emissions (Hung et. al. 2017) or cause higher $N_2O$ emissions (Dörsch et. al. 2022; Müller Júnior et. al. 2019; Olofsson and Ernfors 2022; Thomas et. al. 2017). Since studies come to different conclusions, the subject needs to be assessed even further.

When assessing the climate impact of CC cultivation, carbon sequestration and potential release of $N_2O$ need to be accounted for. Preceding, studies indicate that cover crops may increase $N_2O$ (Mitchell et. al. 2019; Müller et. al. 2019), especially oilseed radish (Olofsson and Ernfors 2022). When cover crops are terminated, organic compounds from the residues might stimulate denitrification and $N_2O$ emissions (Mitchell et. al. 2013). Likewise, the underlying mechanisms behind denitrification during freeze and thaw cycles.

Recently, it has been reviewed that the quality of the crop residue affects the $N_2O$ emissions (Abalos et. al. 2022; Lashermes et. al. 2022). Abalos et. al. (2022) means that a high content of water-soluble C and easily decomposable C increase $N_2O$ emissions. Immature crops usually have a composition with a low C/N ratio due to high N concentration, low cellulose content, high soluble dry matter, and high water-soluble C contents. Cover crops often represent immature crop residues which increase $N_2O$ emissions compared to mature crop residues.

Similarly, regardless of if the plant material was physiologically mature or still green - the content of soluble fractions that was strongly linked to the $N_2O$

emissions (Lashermes et. al. 2022). However, physiologically immature green parts of crop residues contain a higher level of soluble fractions.

## 2.5 Cover Crops – Oilseed Radish, *Raphanus sativus var. oleiformis*, and *Phacelia tanacetifolia*

CCs are used to build up a long term sustainable agricultural system regarding nutrient supply to crops and soil structure. An overall ideal CC does not exist but is dependent on when and where it is grown. OR is part of the *Brassicaceae* family (Aronsson et. al. 2012). As a CC, OR has a large root system with a tap root that improves soil structure (Olsson et. al. 2013). To a depth of 2.5 m down the soil profile, OR efficiently removes N that would otherwise increase the risk of leaching nutrients into aquatic systems (Olsson et. al. 2013). The study concluded that 100 kg N/ha was absorbed in the above ground plant parts. A trial-study over 9 years showed that OR had an annual average of 2.53 Mg C $ha^{-1}$ assimilation from above ground plant parts (Chahal et. al. 2020). OR has an ability to reduce N leaching without an increased risk of phosphorus leaching (Norberg and Aronsson 2018).

During winter, OR is cold resistant down to temperatures of minus 6°C (Aronsson et. al. 2012). In the temperate zone, it is often frost-killed but not always. Negative aspects of OR as a CC is that it has small seeds and is drought sensitive which makes it harder to establish for the producers. But benefits for the producers are that OR has some resistance to diseases. Most varieties of OR are resistant to the soilborne disease *Plasmodiophora brassicae* and show remediating effects against beet cyst nematodes (Aronsson et. al. 2012). Many species, including OR, in the Brassica family contain the secondary metabolite glucosinolates (GSLs) (Bischoff 2021; Wu et. al 2021b).

*Phacelia tanacetifolia* (PH) is an annual crop in the family of *Boraginaceae* (Aronsson et. al. 2012). Recently it has gained attention to be used as a source for pollinators to increase biodiversity. It is not related to the common agricultural crops which decreases the risk of spreading diseases - a benefit for producers. Nevertheless, it is a frost sensitive crop and easily frost killed during cold periods (Aronsson et. al. 2012). Dissimilar to OR, PH does not contain GSLs.

## 2.6 Glucosinolates

Glucosinolates (GSLs) are found in the vacuole of numerous agricultural crops and are a major sulphur component in crucifers, especially the *Brassiceae* family (Buscot and Varma 2005; Yara 2023). GSLs are a part of the plant's defence

mechanism against herbivores and pathogens (Kopriva 2021). GSLs are secondary plant metabolite and organic compounds derived from glucose and amino acid that contain sulphur and nitrogen (Yara 2023). The concentration of GSLs in the plants are normally 14-24 μmol $g^{-1}$ in dry leaves and 55-115 μmol $g^{-1}$ in dry seeds.

GSLs are relatively stable in the plant cell (Barba et. al. 2016). But when the plant tissue containing GSLs is disrupted by cutting, chopping, mixing, or chewing the enzyme myrosinase is released. The enzyme is usually stored in a different cell or a different cellular compartment depending on the plant species. Myrosinase degrades GSLs to a glucose molecule and an unstable aglycone. Through a series of biochemical reactions GSLs are broken down to isothiocyanates (ITCs) or epithionitrile. GSLs breakdown products, especially ITCs, have fungicidal and bactericidal properties. The biochemical pathway of degradation of GSLs has a glucose molecule as the first step (Barba et. al. 2016). Therefore, a glucose solution can be used to mimic the presence of GSLs and simulate a similar effect.

# 3. Hypothesis

The purpose of this master thesis is to find out the underlying process and mechanism behind the $N_2O$ emissions from the cover crop oilseed radish, *Raphanus sativus var oleiformis* (OR), and *Phacelia tanacetifolia* (PH). The hypothesis is that the glucosinolates (GSLs) content in OR provides a carbon source for heterotrophic denitrifiers in the soil. In the degrading process, denitrification - then causes a higher level of $N_2O$ emissions compared to PH which does not contain GSLs. The aim was addressed by testing the following hypothesis:

An addition of glucose (15 g $m^{-2}$) will cause a higher increase of $N_2O$ emissions in PH than in OR.

# 4. Methodology

## 4.1 Cultivation of Crops

The crops OR and PH were cultivated in a greenhouse in Vegetum, Alnarp. In a cultivation period of 5 weeks (17th of February to 15th of April 2022) the crops grew at a minimum temperature of 20°C for 16 hours a day (18°C at night). Artificial light provided using high pressure sodium (HPS) lights. The crops were germinated in sowing soil. Nine days later, the crops were transplanted into 1.5L pots with organic soil. Fertilizer and water were added for optimum growth. After 58 days, the crops were harvested for incubation.

## 4.2 Biomass Sampling

Samples of leaves and roots were taken 4th of April for analysis of C/N ratio and dry weight biomass yield. For analysis of total nitrogen and carbon content of OR and PH biomass, 6 randomized leaves and 1 root were selected for each crop. The plant material was dried in 60°C for 48 hours. The dried material was cut into pieces of 1-2 cm and mixed for homogenous structure. The plant material was grinded using a ball mill for 4 minutes at 30 rounds per minute. Afterwards, the plant material was incubated at 60°C for 2 hours to obtain completely dry samples. Using the Mettler Toledo scale, 5 mg ($\pm$0.5) was weighed. The total nitrogen and carbon content was analyzed on Flash 2000 (Organic Elemental Analyzer).

For dry weight biomass yield, 6 randomized leaves and 1 root of OR and PH were selected. The roots were washed to remove soil and weighed. Next, the leaves and roots were dried at 60°C for 48 hours and weighed again.

## 4.3 Soil Sampling

Soil was collected from SITES Lönnstorp Research station on the 4th and 7th of April to determine C/N ratio, pH, and water content (g $H_2O$ $g^{-1}$ soil $dw^{-1}$) of the soil. Soil was collected at 0-10 cm depth from 3 undisturbed sites. Soil collected on the 7th of April was dried at room temperature for 48 hours due to high water content. The soil was sieved at 4 mm. Afterwards, the soil was pre-incubated at room temperature for 12-14 days.

For analysis of the total nitrogen and carbon content of the soil, samples were air dried at room temperature overnight. The soil was sieved at 1 mm. Afterwards, the soil was grinded using a ball grinder for 4 minutes at 30 rounds per minute. The soil was incubated at 60°C for 2 hours. Using the Mettler Toledo scale, 30 mg ($\pm$0.5) was weighed. The total nitrogen and carbon content was analyzed on Flash 2000 (Organic Elemental Analyzer).

The pH was measured according to the European standard with a 1:5 ratio (v/v) using MilliQ water (SIS 2022). For determination of water content (g $H_2O$ $g^{-1}$ soil $dw^{-1}$), soil sample was weighed, dried at 105°C overnight and weighed once more.

## 4.4 Experimental Design

Plant material was prepared with soil from SITES Lönnstorp Research station in 30 metallic cylinders. According to a scheme, plant parts of OR and PH were separated by leaves - aboveground plant material (A), roots - belowground plant material (B), leaves and roots - aboveground plus belowground (AB) and leaves and roots with added glucose solution - aboveground, belowground plus glucose solution (ABG+). Control samples with only soil and soil with glucose solution (G+C) were prepared as well. Soil and plant material was prepared in 3 blocks with 3 replicates and exposed to freezing treatment.

Soil used was from SITES Lönnstorp Research Station with content of 22% clay and 3.2% organic material (Hansson et. al. 2021). In 400 $cm^3$ cylinders, 182.0 g of dry weight (dw) soil was placed. The soil was prepared with a bulk and compact density with respectively 1.30 and 2.65 g $cm^{-3}$ with 14 g $H_2O$ $g^{-1}$ soil $dw^{-1}$, see table 1. To obtain 60% water filled pore space, 17.5 ml of deionized water was added on top. Soil and deionized water were added in two stages: half and half on top. In treatments with added glucose of OR, PH, and control with soil (ABG+O, ABG+P, and G+C), 15.0 g $m^{-2}$ glucose was added per area (Gilliam et. al. 2008). The added amount of glucose was based on Gilliam et. al. (2008) which had a range between 24.5 to 12.3 g $m^{-2}$.

Plant material was prepared by separating leaves and roots from stem. Roots were washed and dried. Leaves and roots were cut to obtain the smallest amount of incision to decrease the risk of GSLs degrading (fig 1). Dry weight of the plant was determined based on a field experiment by Olofsson and Ernfors (2022) in SITES Lönnstorp Research station, 127 and 124 g $m^{-2}$ of OR and PH respectively. The dry weight plant material was scaled down to the area of the cylinder, 14 $cm^2$.

The fresh weight of the plant material was weighed according to table 1 based on the measured water content of 88% of OR and PH. The ratio between A and B of plant material was based on an estimation of the dw ratio of roots and the whole crop (OR - 4.8% and PH - 1.3%) to mimic field conditions. Finally, the samples were placed in a freezer (-26.7°C) for 96 hours. The cylinders were prepared and incubated blockwise.

*Table 1. Soil and crop variables. Determined soil variables of dry weight, bulk density, compact density, soil water content, water filled pore space (WFPS), and added glucose. Mean values of crop variable dry weight biomass, gram per square metre. C= control, AO= aboveground oilseed rape, AP= aboveground Phacelia tanacetifolia, BO= belowground oilseed rape, and BP= belowground Phacelia tanacetifolia.*

| Soil Variables | | | Value | | |
|---|---|---|---|---|---|
| Dry weight (g) | | | 182 | | |
| Bulk density (g $cm^{-3}$) | | | 1.30 | | |
| Compact density (g $cm^{-3}$) | | | 2.65 | | |
| Soil water content (g $H_2O$ $g^{-1}$ soil $dw^{-1}$) | | | 0.14 | | |
| Water filled pore space (%) | | | 60.0 | | |
| Added glucose (g $m^{-2}$) | | | 15.0 | | |
| Crop Variables | C | AO | AP | BO | BP |
| Dry weight biomass (g $m^{-2}$) | n/a | 121 | 122 | 6.10 | 1.61 |

*Figure 1. Plant material of OR and PH. Added on top of the soil (Annika Swensson Källén, April 2022).*

## 4.5 Incubation of Samples

For incubation, samples were stored in a dark cabinet for 35 days at room temperature. Temperature was noted with each gas measurement using a wireless sensor. Samples were placed blockwise in a randomized order. The weight was noted on 7 occasions during this period every fifth day.

On the 22nd day - samples were placed in the freezer for another 96 hours. After 48 hours in the freezer, 1.5 ml deionized water/0.08 mM glucose solution was added. After added deionized water or glucose solution, samples were placed back in the freezer for 48 hours. Afterwards, samples were incubated for 14 days at room temperature.

## 4.6 Gas Measurement

Gas measurement of $N_2O$, $CO_2$ and $CH_4$ was studied during a period of 35 days. Gas samples were assessed on 19 occasions - from 19th of April to 23rd of May. The first day, gas measurement was done twice with a short interval between the first and second measurement. First time, all 3 blocks of samples were measured simultaneously directly after taking the samples out of the freezer. Afterwards, one more gas measurement was done blockwise the same day. After 18 days of incubation, the samples were exposed to a second freeze treatment of 96 hours in the freezer. The first day after the second freeze treatment, gas measurement was done twice with a short interval in between during the first 24 hours. Afterwards, gas measurements were taken for another 12 days. Before each measurement, the temperature was noted.

The gas measurement procedure was taken in intervals of 60 minutes. Two samples per cylinder were taken, one at time zero ($T_0$) and one after 60 minutes ($T_{60}$). The gas measurements were done per block. A 10 ml syringe was used to extract 9 ml of gas into a 5.9 ml vial. The vials had been evacuated beforehand. For each measurement, the samples were isolated using an airtight glass jar with a membrane on top for the syringe (fig 2). According to a time schedule, the cylinders were placed in the glass jar and closed. The syringe was placed through a membrane on top and calibrated 3 times per jar by pushing 10 ml of air in and out without removing the syringe from the membrane. After calibration, the sample was taken and collected in vial. Samples then were analyzed on a gas chromatograph to determine concentrations of primarily $N_2O$, as well as $CO_2$ and $CH_4$.

*Figure 2. Experimental setup (Annika Swensson Källén, May 2022).*

## 4.7 Statistical Analysis

Cumulative emissions for $N_2O$, $CO_2$ and $CH_4$ were calculated by linear interpolation of emission values between sampling dates, for each 30 cylinders. Mean cumulative emissions of $N_2O$ and $CH_4$ for each treatment were also converted into $CO_2$-eq. using GWP100 from Nabuur et. al. (2022) for comparison of climate impact. For $N_2O$ and $CH_4$, 1 kg of $CO_2$ is 273 and 25 kg respectively.

The study compared the mean cumulative values of $N_2O$, $CO_2$ and $CH_4$, as well as the mean values of the crop C/N ratio, between treatments using ANOVA in the R programming language, specifically in RStudio version 2022.12.0+353. Prior to analysis, all data underwent normality and homoscedasticity checks of the residuals using Shapiro's test and Quantile-Quantile Plots (qqnorm). A logarithmic transformation was applied to $N_2O$ and $CO_2$ to obtain normal distributions, with a constant value of 10 added to the former to remove negative values. For data that exhibited significant differences in ANOVA, compact letter display (cld) and Friedman's test was conducted. The same method was used to compare CC's and glucose treatment, with a constant addition of 5.

# 5. Results

## 5.1 Soil and Crop Variables

Values for soil variables are presented in table 2. The soil mean C/N ratio was 10.4 (± (standard error) 0.21) with mean value pH of 7.21 (± 0.01). The air temperature during the study period was an average of 18.1°C (± 0.77).

The crop C/N ratios are all presented in table 2 below. The control had no plant material thus no C/N ratio. AO, AP, BO, and BP had a C/N ratio of 26.9, 17.7, 30.5, and 35.3 respectively.

*Table 2. Result of soil and crop variables. Mean values (and standard errors) of the soil variables dry weight, C/N ratio, pH, water-filled pore space (WFPS), bulk density, compact density, soil water content after thawing, added glucose and air temperature. Mean values (and standard errors) of crop variable C/N ratio. C= control, AO= aboveground oilseed rape, AP= aboveground Phacelia, BO= belowground oilseed rape, and BP= belowground Phacelia. The letters in superscript indicate significant differences between treatments. If two treatments share the same letter, they are not significantly different.*

| Soil Variables | | | Values | | |
|---|---|---|---|---|---|
| C/N Ratio | | | 10.6 (0.21) | | |
| pH | | | 7.21 (0.01) | | |
| Air temperature (°C) | | | 18.1 (0.77) | | |
| Crop Variables | C | AO | AP | BO | BP |
| C/N Ratio | n/a | 26.9(1.79)$^{a}$ | 17.7(0.06)$^{b}$ | 30.5(0.00)$^{c}$ | 35.3(0.14)$^{d}$ |

## 5.2 $N_2O$ Emissions

During the first study period, emissions of $N_2O$ were low the first two measurements but after 24 hours all treatments increased except control with added glucose, fig 3. Aboveground Phacelia showed a latter peak flux at the end of the first study period.

Mean cumulative emissions (and standard error bars) of $N_2O$-N is presented in figure 4 below. The mean cumulative $N_2O$-N emission were from highest to lowest AP 135.3 mg $m^{-2}$ $32d^{-1}$ (± 101), ABP 44.3 mg $m^{-2}$ $32d^{-1}$ (± 48), ABG+O 43.9 mg $m^{-2}$ $32d^{-1}$ (± 14), ABG+P 30.7 mg $m^{-2}$ $32d^{-1}$ (± 23), ABO 27.5 mg $m^{-2}$ $32d^{-1}$ (± 17),

AO 23.8 mg $m^{-2}$ $32d^{-1}$ (±12), BP 16.7 mg $m^{-2}$ $32d^{-1}$ (± 11), BO 11.4 mg $m^{-2}$ $32d^{-1}$ (± 12), C 5.18 mg $m^{-2}$ $32d^{-1}$ (± 12) and G+C 4.45 mg $m^{-2}$ $32d^{-1}$ (± 2).

Scaled up to field conditions, mean cumulative emissions of $N_2O$-N were for ABG+O 0.44, ABG+P 0.31, ABO 0.27, ABP 0.44, AO 0.24, AP 1.35, BO 0.11, BP 0.17, C 0.05, and G+C 0.05 in kg $hectare^{-1}$ $32d^{-1}$.

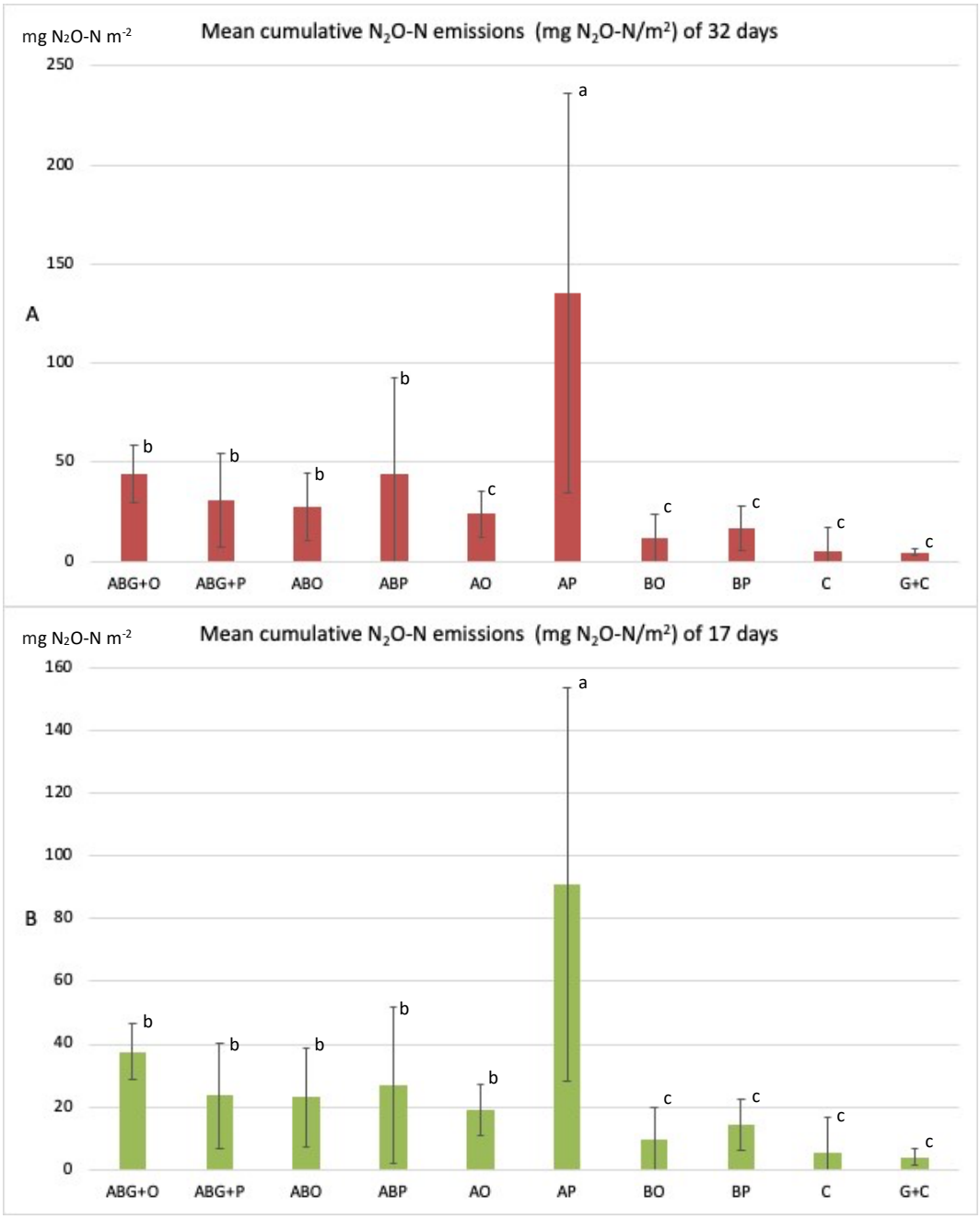
In the first study period of 17 days, the mean cumulative emissions of $N_2O$-N are presented in figure 4. The mean cumulative emissions were in descending order AP 90.9 (± 63), ABP 26.9 (± 25), ABG+O 37.6 (± 9), ABG+P 23.5 (± 17), ABO 23.1 (±16), AO 18.9 (± 8), BP 14.1 (± 8), BO 9.72 (± 10), C 5.31 (± 11) and G+C 3.91 (± 3) mg $m^{-2}$ $17d^{-1}$.

Second study period of 10 days is presented in figure 4. The mean cumulative emissions of $N_2O$-N were in descending order AP 40.6 (± 40), ABP 16.0 (± 21), ABG+P 7.05 (± 8), ABG+O 5.75 (± 5), AO 4.74 (± 3), ABO 4.09 (± 5), BP 2.20 (± 2), BO 1.08 (± 3), C 0.02 (± 0.3) and G+C 0.79 (± 3) mg $m^{-2}$ $10d^{-1}$.

During the full study period, mean cumulative emissions of $N_2O$-N were higher in AP compared to all other treatments ($p<0.05$). Mean cumulative $N_2O$-N emissions were higher ABO, ABP, ABG+O and ABG+P compared to AO, BO, BP, G+C and C ($p<0.05$). G+C showed the lowest mean cumulative emission of $N_2O$-N by all treatments. The first study period showed similar results but there were no significant differences between the treatments in the second study period. Comparison between glucose and no added glucose between CC treatment showed no significant difference ($p<0.05$).

When converted into $CO_2$-eq., cumulative $N_2O$-N emission corresponded to 12.0, 8.38, 7.51, 12.1, 6.50, 36.9, 3.11, 4.56, 0.01, and 0.01 g $m^{-2}$ $32d^{-1}$ for ABG+O, ABG+P, ABO, ABP, AO, AP, BO, BP, C, and G+C respectively.

*Figure 3. Timeline of N2O-N emissions. Emissions of $N_2O$-N evolved (μg $m^{-2}$ $h^{-1}$) for each treatment during the first study period (A) of 17 days, and second study period (B) of 10 days. C= control, AO= aboveground oilseed rape, AP= aboveground Phacelia, BO= belowground oilseed rape, BP= belowground Phacelia, ABO= aboveground+belowground oilseed rape, ABP= aboveground+belowground Phacelia, ABG+O= aboveground+belowground+added glucose oilseed rape, ABG+P= aboveground+belowground+added glucose Phacelia, and G+C= control with added glucose. Most diverse standard error bars are presented - figure A presents AP, ABG+P, and ABG+O. Figure B presents AP and ABP.*


mg N2O-N m-2
Mean cumulative N2O-N emissions (mg N2O-N/m2) of 32 days
250
200
150
A
100
50
0
a
b
b
b
b
c
c
c
c
c
ABG+O
ABG+P
ABO
ABP
AO
AP
BO
BP
C
G+C
mg N2O-N m-2
Mean cumulative N2O-N emissions (mg N2O-N/m2) of 17 days
160
140
120
100
B
80
60
40
20
0
a
b
b
b
b
b
c
c
c
c
ABG+O
ABG+P
ABO
ABP
AO
AP
BO
BP
C
G+C

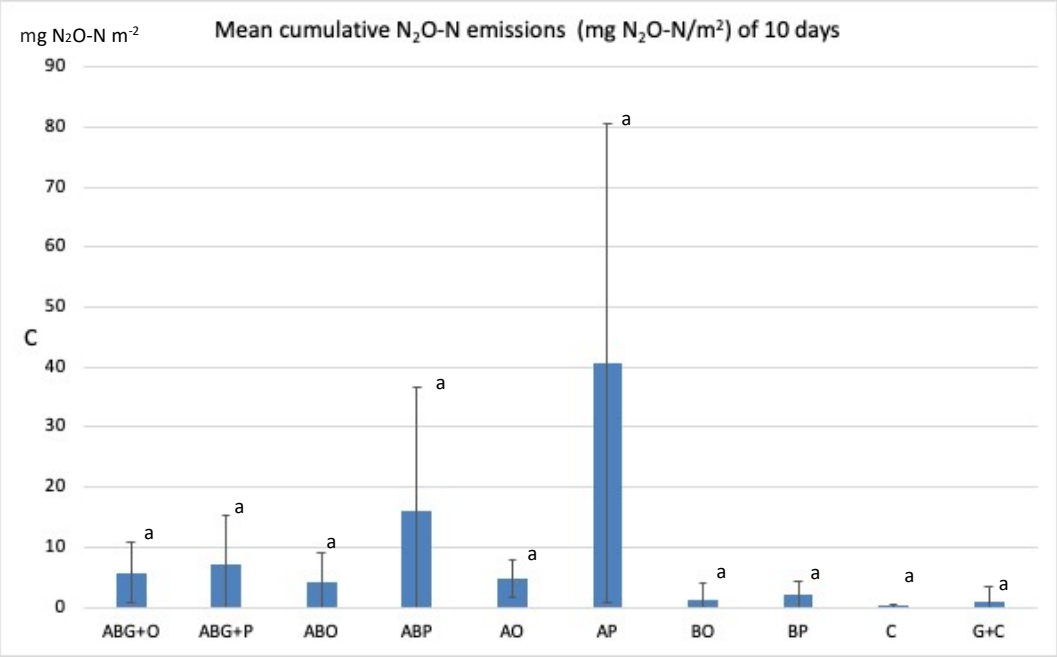



*Figure 4. Cumulative N2O-N emissions. Mean cumulative emissions of $N_2O$-N evolved (mg $m^{-2}$) (and standard error bars) for each treatment during the full study period (A), the first (B) and second study period (C). C= control, AO= aboveground oilseed rape, AP= aboveground Phacelia, BO= belowground oilseed rape, BP= belowground Phacelia, ABO= aboveground+belowground oilseed rape, ABP= aboveground+belowground Phacelia, ABG+O= aboveground+belowground+added glucose oilseed rape, ABG+P= aboveground+belowground+added glucose Phacelia, and G+C= control with added glucose. The letters in superscript indicate significant differences between treatments. If two treatments share the same letter, they are not significantly different.*

## 5.3 $CO_2$ Emission

Throughout the study period, $CO_2$ showed higher emissions in OR and PH with ABG+, AB and A compared to controls and OR and PH with only B, see fig 5. During the first period, the emissions formed an S curve of the OR and PH with ABG+, AB and A treatments. During the second study period, OR with ABG+, AB and A showed a peak after 24 hours, see figure 5.

Presented in figure 6, the mean cumulative $CO_2$ emission during the study period were from highest to lowest AO 238 mg $m^{-2}$ $32d^{-1}$ (± 38.1), ABO 237 mg $m^{-2}$ $32d^{1}$ (± 38.4), ABG+O 235 mg $m^{-2}$ $32d^{-1}$ (± 41.1), ABP 186 mg $m^{-2}$ $32d^{-1}$ (± 9.49), ABG+P 175 mg $m^{-2}$ $32d^{-1}$ (± 20.2), AP 149 mg $m^{-2}$ $32d^{-1}$ (± 5.86), BO 43.0 ug $m^{-2}$ $32d^{-1}$ (± 3.20), C 37.3 mg $m^{-2}$ $32d^{-1}$ (± 1.41), BP 36.9 mg $m^{-2}$ $32d^{-1}$ (± 0.56), and G+C 35.7 mg $m^{-2}$ $32d^{-1}$ (± 2.47).

On the lower end, there was no significant difference in the cumulative emissions of $CO_2$ between C, G+C, AP, BO, and BP ($p<0.05$). There was a significant difference between ABP and ABG+P compared to all other treatments ($p<0.05$).

AO, ABO and ABG+O showed significantly higher cumulative emissions compared to all other treatments ($p<0.05$). In descending order, the emission of $CO_2$ in kg hectare$^{-1}$ 32d$^{-1}$ were AO 2.39, ABO 2,37, ABG+O 235, ABP 1.86, ABG+P 1.75, AP 1.49, BO 0.43, C 0.37, BP 0.37, and G+C 0.36.

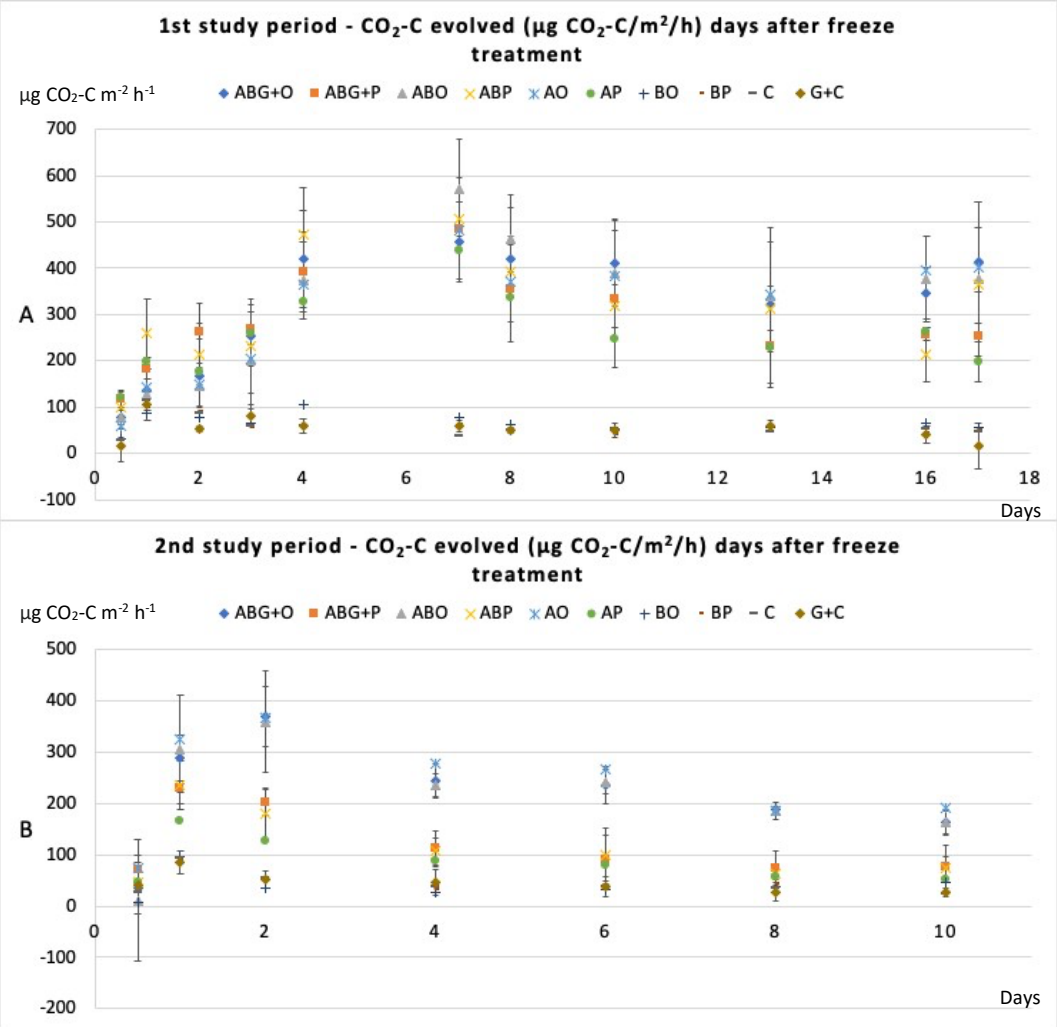



*Figure 5. Timeline of CO2-C emissions. Emissions of $CO_2$-C evolved (ug m$^{-2}$ h$^{-1}$) for each treatment during the first (A) of 17 days, and second study period (B) of 10 days. C= control, AO= aboveground oilseed rape, AP= aboveground Phacelia, BO= belowground oilseed rape, BP= belowground Phacelia, ABO= aboveground+belowground oilseed rape, ABP= aboveground+belowground Phacelia, ABG+O= aboveground+belowground+added glucose oilseed rape, ABG+P= aboveground+belowground+added glucose Phacelia, and G+C= control with added glucose. Most diverser standard error bars are presented, figure A and B presents ABG+O, ABG+P, ABO, ABP, and G+C.*

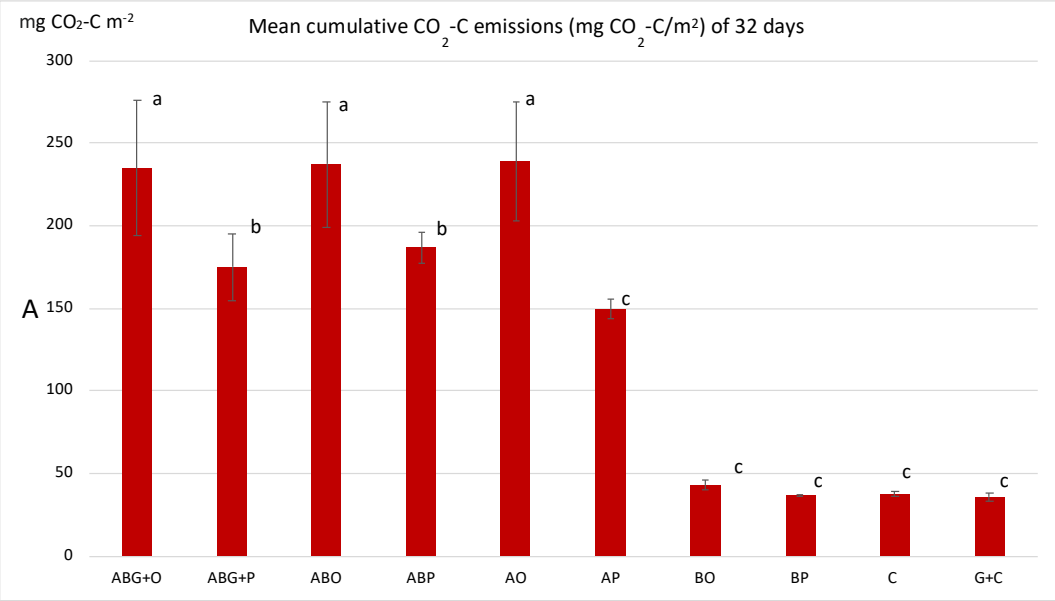



*Figure 6. Cumulative CO2-C emissions. Mean cumulative emissions of $CO_2$-C evolved (mg $m^{-2}$) (and standard error bars) for the full study period of 32 days. C= control, AO= aboveground oilseed rape, AP= aboveground Phacelia, BO= belowground oilseed rape, BP= belowground Phacelia, ABO= aboveground+belowground oilseed rape, ABP= aboveground+belowground Phacelia, ABG+O= aboveground+belowground+added glucose oilseed rape, ABG+P= aboveground+belowground+added glucose Phacelia, and G+C= control with added glucose. The letters in superscript indicate significant differences between treatments. If two treatments share the same letter, they are not significantly different.*

## 5.4 $CH_4$ Emission

Throughout the study period, $CH_4$ emissions were low or negative (fig 7). Fig 7A shows that the $CH_4$ emissions oscillate during the first week of measuring to become more stabilized towards the end. The second study period showed a single peak during the first measurement (fig 7B).

The mean cumulative emissions were ABP -30.9 ug $m^{-2}$ $32d^{-1}$ (± 8.2), BO -23.5 ug $m^{-2}$ $32d^{-1}$ (± 7.3), ABO -21.6 ug $m^{-2}$ $32d^{-1}$ (± 8.6), AP -20.3 ug $m^{-2}$ $32d^{-1}$ (± 6.5), C -19.2 ug $m^{-2}$ $32d^{-1}$ (± 7.7), G+C -18.0 ug $m^{-2}$ $32d^{-1}$ (± 16), BP -17.2 ug $m^{-2}$ $32d^{-1}$ (± 15), AO -15.2 ug $m^{-2}$ $32d^{-1}$ (± 7.8), ABG+P -14.6 ug $m^{-2}$ $32d^{-1}$ (± 8.5), and ABG+O -6.2 µg $m^{-2}$ $32d^{-1}$ (± 7.0), see figure 8. There were no significant differences between the treatments.

When converted into $CO_2$-eq, cumulative emissions of $CH_4$ were equal to an uptake of $CO_2$ of 155 µg, 365 µg, 540 µg, 773 µg, 380 µg, 508 µg, 588 µg, 430 µg, 480 µg, and 450 µg for ABG+O, ABG+P, ABO, ABP, AO, AP, BO, BP, C and G+C respectively.

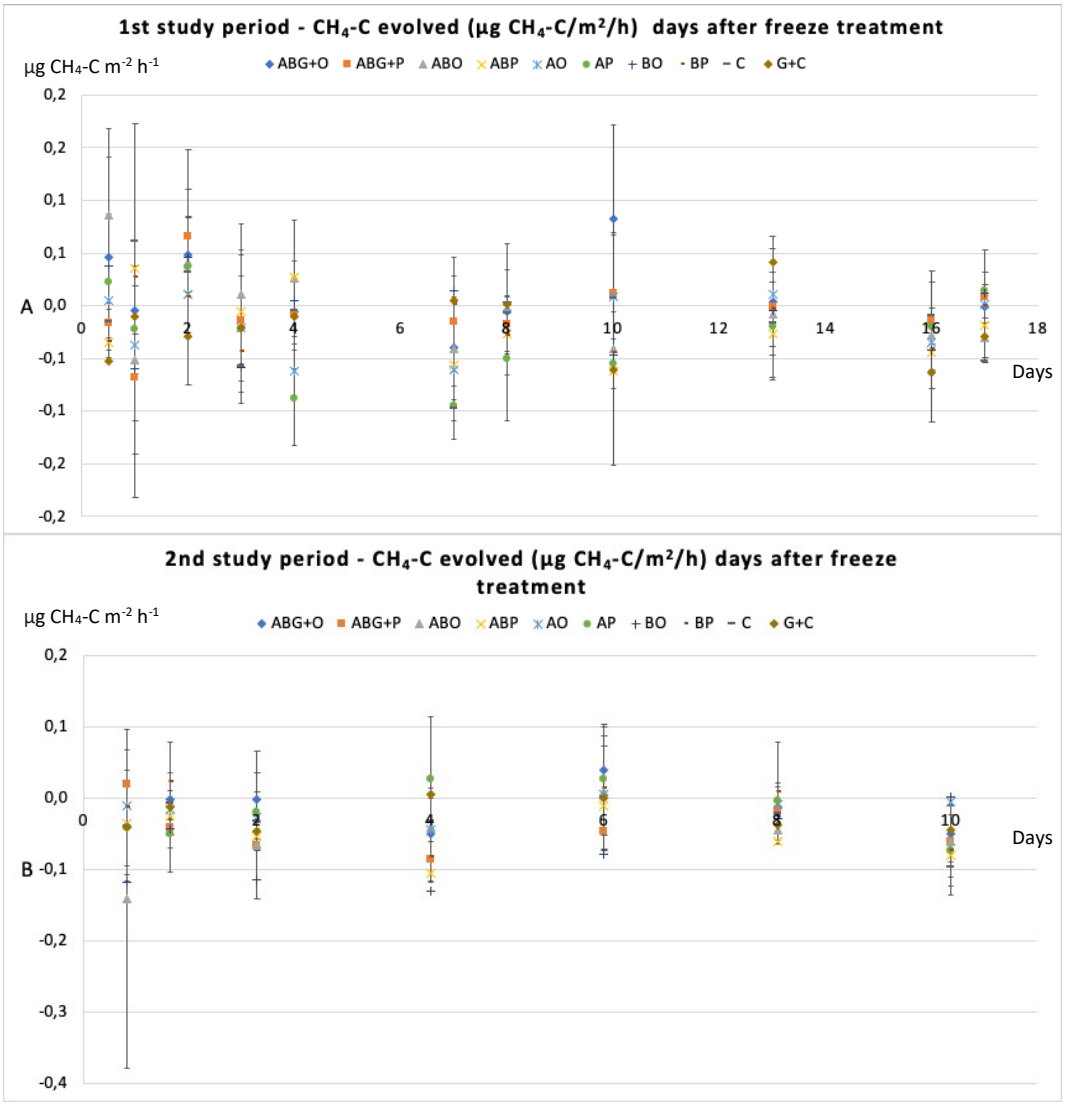



*Figure 7. Timeline of CH4-C emissions. Emissions of $CH_4$-C evolved ($\mu g\ m^{-2}\ h^{-1}$) for each treatment during the first study period (A) of 17 days and second study period (B) of 10 days. C= control, AO= aboveground oilseed rape, AP= aboveground Phacelia, BO= belowground oilseed rape, BP= belowground Phacelia, ABO= aboveground+belowground oilseed rape, ABP= aboveground+belowground Phacelia, ABG+O= aboveground+belowground+added glucose oilseed rape, ABG+P= aboveground+belowground+added glucose Phacelia, and G+C= control with added glucose. Most diverse standard error bars are presented. Figure A and B presents respectively ABG+O, ABG+P, and ABO as well as ABO, ABG+P, and AP.*

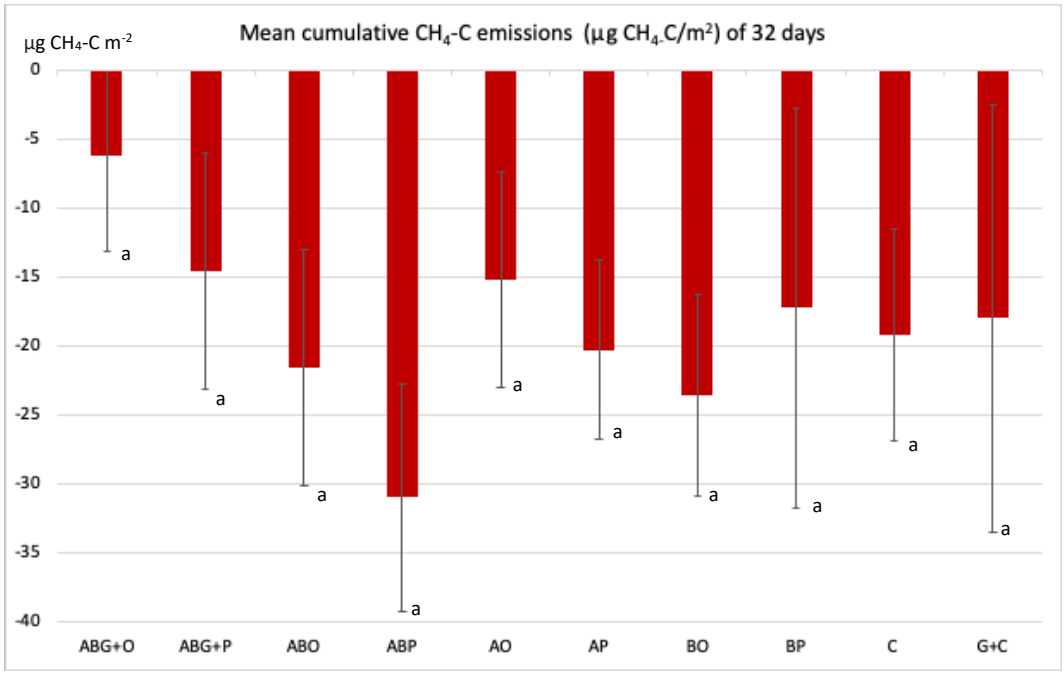



*Figure 8. Cumulative CH4-C emissions. Mean cumulative emissions of $CH_4$-C evolved ($\mu g\ m^{-2}$) for each treatment during the full study period (and standard error bars) of 32 days. C= control, AO= aboveground oilseed rape, AP= aboveground Phacelia, BO= belowground oilseed rape, BP= belowground Phacelia, ABO= aboveground+belowground oilseed rape, ABP= aboveground+belowground Phacelia, ABG+O= aboveground+belowground+added glucose oilseed rape, ABG+P= aboveground+belowground+added glucose Phacelia, and G+C= control with added glucose. The letters in superscript indicate significant differences between treatments. If two treatments share the same letter, they are not significantly different.*

# 6. Discussion

## 6.1 Emissions of $N_2O$

Greenhouse gas (GHG) emission from agriculture stands for 14% of Sweden's total GHG emission (Naturvårdsverket n.y.). In 2021, $N_2O$ emitted from soil stood for 40% of the total GHG emission from agriculture in Sweden. The mechanisms behind it are still not clearly understood and therefore needs to be further investigated.

The primary goal of this study was to identify the underlying process behind $N_2O$ emissions, the CCs oilseed radish, *Raphanus sativus var oleiformis* (OR), and *Phacelia tanacetifolia* (PH) were studied to exemplify differences in outcome. A comparison was made between the aboveground and belowground plant tissues, separate and together and plant tissues together with added glucose. The hypothesis was that OR as a Brassicaceae crop contains glucosinolates which provide a carbon source for heterotrophic denitrifiers in the soil. In the denitrification process, the carbon may cause a higher level of nitrous oxide gas emissions compared to *Phacelia tanacetifolia* which does not contain glucosinolates. As a control, labile C was added in the form of glucose to soil and aboveground and belowground plant material of OR and PH.

The plant material was exposed to a freezing treatment to mimic freeze-and-thaw cycles (FTC) which according to previous studies increases $N_2O$ emissions (Wagner Riddle et. al. 2017). Contrary to the hypothesis the result of this study showed that aboveground PH accumulated significantly higher flux of $N_2O$ emissions compared to all other treatments, a mean cumulative of 135.3 mg $m^{-2}$ $32d^{-1}$ (± 2192) shown in fig 4, which is approximately 360 kg $CO_2$/ha.

Comparison between CC's and glucose treatment appears that added glucose does not affect the CC's emission of $N_2O$. During the second study period, ANOVA showed a significant difference between the treatments ($p<0.05$), but downstream testing of cld and Friedman's test did not. Therefore, the hypothesis in this study cannot be confirmed. In this study, labile C (15 g $m^{-2}$) did not cause a higher increase of $N_2O$ emission in PH than in OR.

The control with soil and soil with added glucose showed as expected the lowest flux of $N_2O$ emissions during this study. There were no significant differences

between the mean cumulative $N_2O$ emissions of the two controls. As previously mentioned, the added amount of glucose was based on Gilliam et. al. (2008) which had a range between 24.5 to 12.3 g $m^{-2}$. An estimated value was chosen to the lower range of 15 g $m^{-2}$ to ensure that the nitrification cycle was not completed so $N_2O$ was transformed to $N_2$. But the added amount of glucose could be too low to make a significant difference in $N_2O$ flux emission since no difference was shown in the results.

Converted to field emission, aboveground PH emitted 1.35 kg $ha^{-1}$ $32d^{-1}$ of $N_2O$-N. The annual $N_2O$-N emission from arable land in Northern Europe is estimated to be between 0-10 kg $ha^{-1}$ according to Hushållningssällskapet (2015). However, according to Wu et. al. (2021a), estimating $N_2O$ emission can lead to over- and underestimation due to inconsistent diurnal $N_2O$ patterns. The many factors influencing $N_2O$ emissions make it difficult to predict.

Previous studies have shown that OR emits significantly larger emissions of $N_2O$ compared to other cover crops (Dörsch et. al. 2022; Müller Júnior et. al. 2019; Olofsson and Ernfors 2022; Thomas et. al. 2017). The significantly higher flux of aboveground PH suggests that OR does not always have the highest $N_2O$ emissions. But OR may be more likely to have high peaks of $N_2O$ flux during FTC.

However, Brown and Morra (2009) suggest that GSLs breakdown products inhibit nitrification. In their study was a greater accumulation of $NH_4^+$ in soil containing high GSLs concentration. During the preparation for this study the plant material was cut, the tissue exudates might have caused myrosinase to hydrolyse GSLs in OR. The GSLs by-products may have caused an inhibition of nitrification which in this study caused a lower $N_2O$ emission compared to PH.

A peak in all treatments was shown 24 hours after freezing treatment during the first period, fig 3. The peak flux of $N_2O$ during the first day could be because of physical and biological changes in soil conditions. FTC induces release of labile C and $NO_3^-$ which drives $N_2O$ emissions - both by fuelling denitrifiers and depleting oxygen (Mørkved et. al. 2006). The slow thawing of the soil could lead to that the denitrifiers became active after 24 hours which led to a peak of $N_2O$ flux (Butterbach-Bahl et. al. 2013; Wagner Riddle et. al. 2017).

Surprisingly, AP showed a later peak flux at the end of the first period. A reason could be that the microbes degrade the longer carbon chains after the labile which leads to a peak flux. However, OR contains a lower percentage of soluble components compared to PH which means it generally has a higher amount of long

carbon chain (Olofsson and Ernfors 2022). The missing peak in OR goes against this theory.

During the second period, all treatments showed a similar pattern as the first. Within the first 24 hours all treatments had a peak flux but afterwards a slow decline. The $N_2O$ fluxes never showed the same height as the first period which can be due to lower availability of C and N.

The ratio between aboveground and belowground plant material were to imitate the field conditions of the ratio between root and shoot. The difference of dry weight (dw) of plant material does not make them comparable. The comparison is therefore focused on the AB treatment compared to AB with added glucose which has an equal amount of dw plant material.

## 6.2 $CO_2$ and $CH_4$ Emission

As GHG with global warming potential, the emission of $CO_2$ and $CH_4$ were determined. Soil respiration is the $CO_2$ produced by microbial activity thus degradation of biomass in the soil (Gyawali et. al. 2019). Added glucose resulted in lower soil respiration in PH, but not in OR. The GSLs concentration in OR could be an explanation. GSLs are biologically inactive molecules, but after tissue disruption they are hydrolyzed by the enzyme myrosinase to several byproducts like indoles, isothiocyanates and nitriles (Omirou et. al. 2010). The hydrolysis products of GSLs constitute a part of the plant's defense mechanism.

Glucose was used to mimic increases in soil carbon availability - in this experiment GSLs. Glucose can be used to mimic soil carbon availability during natural processes such as root exudation or litter decomposition (Zhou et. al. 2021). Zhou et. al. (2021) found that the addition of glucose leads to a change in the microbial community, and as the amount of glucose increases, the range of bacterial communities that are affected also increases more. OR's natural components of GSLs may cause an additive relationship of soil respiration with the added glucose. Glucose added on top of GSLs, caused a higher soil respiration.

In this study, raw data of $CO_2$ showed negative values. $CO_2$ emissions should always be positive unless photosynthesis occurred. A possible reason could be an unnoticed weed in the incubation. Or it could be an error that occurred during the gas chromatography (GC). The measurement of $T_0$ and $T_{60}$ could be reversed and therefore causing a negative value. Since it is impossible to know the reason behind, the negative data was not removed.

The accumulated emission of $CH_4$ was negative, figure 8. Throughout the study period the $CH_4$ did not show any significantly high or low peaks, see figure 7. The uptake of $CH_4$ was low compared to $N_2O$, μg compared to kg in $CO_2$-eq. But comparing total emission, $CH_4$ had a positive impact in terms of the global warming potential. The consumption or low emission of $CH_4$ was an anticipated result and goes in line with Topp and Pattey (1997) since the soil conditions were anaerobic. In 2021, $CH_4$ represented 49% of the total GHG emission in Sweden's agricultural sector but derived from animal feed digestion, not soil (Naturvårdsverket n.y.).

## 6.3 Crop Variables Influencing $N_2O$ Emissions

$N_2O$ emission is affected by many factors such as soil type, temperature, pH, and moisture (Wu et. al. 2021). These environmental factors were reduced in this study since it was not set in a field but a laboratory. Nevertheless, the difference between the treatments were the crop type, C/N ratio and amount of aboveground and belowground plant tissues. The different amount of biomass between OR and PH affects the $N_2O$ emission. To reduce these factors, the discussion is focused on CC with AB plant material compared to CC AB with added glucose.

The different dry weight biomass affects physiological factors and not only the building blocks of C and N for the microbes to consume. A lower amount of biomass dries out faster which affects the amount of water in the soil. The dissimilar water availability between treatments may alter the $N_2O$ emission.

The dry weight biomass in grams per square meter of plant material of OR and PH was based on Ernfors and Olofsson (2022) to mimic field conditions. The amount of OR belowground plant material was significantly higher than PH. The C/N ratio in OR aboveground plant material was significantly higher than PH, 26.1 compared to 17.7 (table 2). The C/N ratio of the belowground plant material of the PH was higher than OR, 35.3 compared to 30.1 (table 2). The factors of dry weight biomass and C/N ratio (table 2) affect $N_2O$ flux emission.

Studies on PH and its effect on $N_2O$ emission is limited. Contrary to recent studies (Ernfors and Olofsson 2022) the leafy part of PH emitted higher levels of $N_2O$ compared to OR (figure 4). The aboveground plant tissue of the PH had the lowest C/N ratio of 17.7 compared to other plant tissues in this study (table 2). Abalos et. al. (2022) means that a lower C/N ratio than 20-30 causes N mineralisation due to their high N concentration. The mineralized N could provide a source of $NH_4^+$ and $NO_3^-$ for denitrifying bacteria which could explain the high $N_2O$ emission of aboveground PH with its low C/N ratio. For further studies, it would be interesting

to increase the source of N in PH to investigate if the $N_2O$ emission increased. Thus, if N is a limiting factor in $N_2O$ emission could be discussed.

The phenotype between PH and OR differentiates. The belowground plant tissue of PH is a fibrous root system with aboveground plant tissues as deeply lobed leaves shown in figure 1 (Aronsson et. al. 2012). OR has belowground plant tissue as a fast-growing tap root and aboveground dentated leaves (Aronsson et. al. 2012). The deeply lobed leaves of PH cause a larger contact surface with the soil. A large contact surface between soil and leaves could mean that more $N_2O$ is formed in the interface. The phenotype of the leaves could explain the high emission of $N_2O$ by AP.

The different volumes of A and B indicates that $N_2O$ emissions do not have an additive relationship. AP measured significantly higher mean cumulative emission of $N_2O$ compared to ABP with no additive pattern. All replicates of AP showed the same pattern between the 3 blocks. This study also highlights the spatial and temporal $N_2O$ emission.

## 6.4 Microbial Activity

The emission of $CO_2$ is an indication of the soil respiration and therefore the microbial activity (Gyawali et. al. 2019). Microbial respiration, $CO_2$ production, is a general process which many microbes catalyze. $N_2O$ and $CH_4$ are more specialized microbial processes (El-Hawwary et. al. 2022). $CH_4$ emission is more prone under an anaerobic environment. $N_2O$ by nitrifiers or denitrifiers under oxic and anoxic conditions. The fact that $CO_2$ and $N_2O$ emission did not show similar patterns during the first study period could be explained by the specialty of $N_2O$ emission.

Interestingly, the different treatments with OR shows higher soil respiration compared to PH, see fig 5. First period, fig 5A, OR and PH shows a similar pattern of $CO_2$ emission. During the second study period, all treatments with OR, except with only belowground plant material, shows higher emission than PH, fig 5B. The mean cumulative emission of $CO_2$ is also higher in OR than in PH, fig 6. A higher soil respiration and microbial activity in OR compared to PH could be due to the preparation of the plant material. The cutting was done with minimized incision the natural formation of the leaves and roots creates a larger cut surface in OR. The larger surface cut might have created more cellular exudates and therefore a higher concentration of degradable tissue litter.

However, according to Omirou et. al. (2013) tissue disruption of *Brassica* species, GSLs are hydrolyzed by myrosinase to ITCs. This compound is toxic to microbes. The soil respiration is higher in PH in the beginning of the study which could be due to the toxicity of ITCs. The observed low soil respiration rate increased in both OR and PH which may be the cellular decomposition of dead microbes (Omirou et. al. 2013). The 24 hours peak the second study period could be due to FTC and the release of substrates.

## 6.5 Methodological Considerations

The presumption for the statistical analysis was that the sample pool was randomized and representative, normally distributed and a homogenized variance of the residuals. As previously mentioned, $N_2O$ exhibits significant spatial and temporal variability which can compromise the normal distribution. This study showed a variability between the replicates. To enhance the data's reliability, it is recommended to increase the number of replicates.

The statistical analysis showed a significant difference of $N_2O$ flux between the treatments in ANOVA ($p<0.05$). The data were transformed to a logarithmic scale with a constant of 10. Although a value lower than 10 compromised normal distribution in Shapiro's test, which indicates that N2O does not always have a normal distribution with its spatial and temporal fluxes. Since ANOVA and Friedman's test results in significant differences there is an indication that there is a normal distribution of $N_2O$ emissions. Therefore, a set constant of 10 was chosen to obtain normal distribution of $N_2O$ flux.

Modeling and scaling up of $N_2O$ emission can be a complex task as it requires considering various factors such as soil properties, water availability, microbial activity, and N availability losses to name a few (Wu et. al. 2015). The methodology of this study would be optimized by having the same amount of plant material belowground and aboveground. Currently, the CC treatments with separate above- and below-ground material are not comparable. Between emissions of $N_2O$, $CO_2$ and $CH_4$ there is no significant difference between the controls and treatments with belowground plant material. An explanation could be too low of the amount of root material throughout the study to affect the outcome.

However, in this study the results show a significant difference between the mean cumulative $N_2O$-N emission between AP and ABP (fig 4). AP showed the highest flux of $N_2O$ which was surprising. The result is an indication that the quantity of the belowground plant material may affect the emissions. This is strengthened by the fact that the indicator of soil respiration, $CO_2$ emission, had a significant

difference between AP and ABP. ABP had higher soil respiration compared to AP. The relatively small amount of belowground root material, 6.10 and 1.61 dw g of OR and PH respectively, may have affected the outcome. Further studies with equal amounts of dw g between aboveground and belowground is recommended to be able to draw a conclusion.

A rationale could be the increasing of the C/N ratio to decrease the $N_2O$ emission during propagation of crops when FTC are common. For a sustainable crop production, producers are recommended to use CCs to mitigate the effects of climate change by carbon sequestration. Propagation of CCs during winter where FTC are common, especially in the south of Sweden, $N_2O$ emissions needs to be taken into consideration. To optimize the use of CCs, producers could consider increasing the C/N ratio of the soil by addition of carbon rich material. Zhang et. al. (2015) means that straw significantly increases C/N ratio. Therefore, incorporating straw when propagating CCs could possibly decrease the emission of $N_2O$ and contribute to a more sustainable food production.

# 7. Conclusion

Contrary previous studies, OR did not show highest N2O emission in this study. Surprisingly, aboveground plant material of PH showed highest emissions throughout the study. The result could not confirm the hypothesis since added glucose did not affect the N2O emission. In further studies, it is recommended to increase the number of replicates and optimize the methodology to investigate the underlying mechanisms behind N2O emissions in the CCs OR and PH. A suggestion for optimizing the methodology of the study would be to acquire the

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# Popular science summary

Every farmer wants a sustainable food production. Farmers are often presented in social media and news as non-sustainable garding the extended use of water or methane burping cows. In fact, sometimes crops must be watered a lot to be able to get a harvest and sometimes cows do burp. Farmers are often presented with sustainable alternatives to decrease the ecological footprint of their production. New findings show that the use of so-called cover crops captures carbon dioxide from the atmosphere, also called carbon sequestration (Aronsson et. al. 2012). But cover crops have plenty environmental benefits and are not cultivated to be harvested.

Oilseed radish and *Phacelia tanacetifolia* are cover crops with different purposes. Oilseed radish is grown because the crop is good at taking up nitrogen, therefore preventing nitrogen from leaching into our seas. *Phacelia tanacetifolia* is grown as a nectare source for pollinators. But when oilseed radish is grown overwinter it has been discovered that it emits nitrous oxide, a greenhouse gas with 273 times more powerful heat trapping effect compared to carbon dioxide (Nabuur et. al. 2022). Nitrous oxide is produced by from microbes in the soil as a part of the nitrogen cycle.

A study was made where the causes of this high nitrous oxide was investigated since the mechanism behind is not very well known. A theory was that oilseed radish contains compounds called glucosinolates which could provide a feed source of carbon for microbes in the soil which could then explain the greenhouse gas emission. However, in a comparison between oilseed radish and *Phacelia tanacetifolia*. However, the theory could not be confirmed that it was glucosinolates that affected the nitrous oxide emission.

However, the study did show surprising results. *Phacelia tanacetifolia* showed higher emissions compared to oilseed radish which has not been shown before. One theory behind this unexpected result was that the leaves of *Phacelia tanacetifolia* had a low ratio between carbon and nitrogen compared to the leaves of oilseed radish. A 20-30 C/N ratio reduces the nitrous oxide emission (Abalos et. al. 2022).

In this case, *Phacelia tanacetifolia* had a 18 C/N ratio, while oilseed radish had a 27 C/N ratio.

This could mean that if farmers put carbon rich plant material on the field when growing cover crops, the emissions of nitrous oxide might not be reduced. One solution could be to spread out straw on the field (Zhang et. al. 2015). With this, we could be one step closer for a sustainable food production.

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