Nitrous oxide (N_2O) emissions from an oilseed radish (*Raphanus Sativus* L.) cover crop frost-killed at different ages

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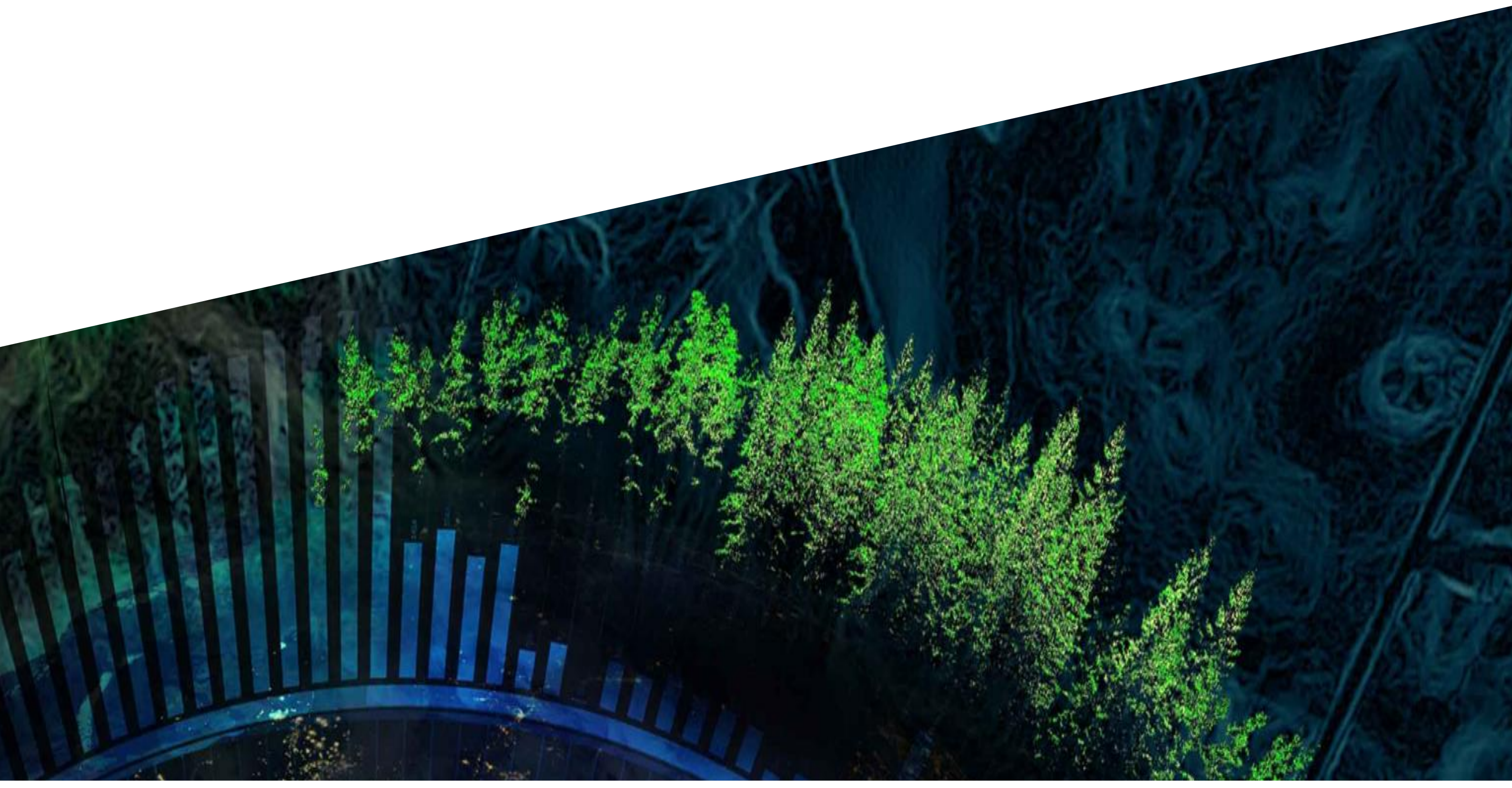

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Abstract

Addressing the urgent need to adapt and mitigate climate change, a significant focus has been placed on reducing greenhouse gas emissions from agriculture. The cultivation of cover crops is an important agricultural practice, offering a multitude of benefits. These crops are meticulously selected based on the farmer's goals, the main crops cultivated, soil conditions, and the local microclimate. In the Scandinavian context, oilseed radish (*Raphanus Sativus* L.), is a popular option, due to it fast growth, deep taproot and termination by frost. While environmental advantages have been well-documented, concurrent greenhouse gas measurements of oilseed radish and its residues have revealed a potential increase in emissions. Given the growing consensus on mitigating anthropogenic N_2O emissions, further investigation into oilseed radish as a cover crop is warranted. Such research is imperative to weigh its benefits against its drawbacks and to derive strategies for effective mitigation measures. This thesis aim was to find out the relationships between N_2O emission and different ages of frost-killed oilseed radish with a focus on the C:N ratios of the plant material. To address this objective, the hypothesis that the crop age of oilseed radish has an inverse relationship with N_2O emissions was tested. Oilseed radish was cultivated in the greenhouse for the purposes of the incubation experiment. The cultivation period lasted 35 days. The three different ages of oilseed radish mentioned, refer to plant material harvested on three specific dates. Age 1 corresponds to plants grown for 21 days and harvested on the 21st day of the cultivation period. Age 2 represents plants grown for 28 days and harvested on the 28th day, while Age 3 indicates plants grown for 35 days and harvested on the 35th day. Subsamples of oilseed radish from each harvest were prepared and analysed for dry matter (DM) content, Total C and Total N. Additionaly, subsamples (6.7g) from each harvest, were randomly selected, packed in zip-lock plastic bags and put in the freezer for the incubation experiment. The soil used for both cultivation and incubation was obtained from soil sampling, air-dried, sieved, and crushed. Subsamples of the soil were taken for further analyses, including total C, total N, and pH measurement. The remaining soil was stored in a cold storage room for the incubation experiment. For soil packing in the incubation cylinders, soil water content was determined gravimetrically, and water was added to achieve 60% WFPS. The experimental setup involved four treatments: cylinders with only soil (control) and soil with oilseed radish frost-killed at three different ages (Age 1, Age 2, Age 3, and Csoil). Each treatment was replicated four times in a completely randomized design. Incubation occurred for 7 days in a dark cabinet at room temperature, followed by sampling and subsequent analyses to quantify N_2O, CO_2, and CH_4 fluxes. Results showed that N_2O emissions in the 3 different ages significantly differ from the control treatment. N_2O fluxes per dry weight, showed that N_2O emissions from oilseed radish frost-killed are notably higher in Age 1 and that can be related with the lowest C:N ratio (6) from the three treatments in the experiment and significantly high emissions. Interestingly, Age 2 didn't appear to have higher emissions as expected. About carbon dioxide CO_2, emissions were higher in all of the treatments in comparison with the control. CO_2 also used as an indicator of the activity of heterotrophs. CH_4 emissions showed no significant differences between treatments. It shows that frost-killed oilseed radish can

enhance N_2O emissions and other factors except from the C:N ratio should be explored for a better understanding of N_2O in different ages of oilseed radish. This study indicates the intricate relationship between the age of frost-killed oilseed radish, its biochemical composition, and the surrounding environmental conditions. The unpredictability of these interactions necessitates further research to accurately determine greenhouse gas emissions from oilseed radish at various ages.

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Abbreviations

N	Nitrogen
N_2O	Nitrous Oxide
C	Carbon
CO_2	Carbon Dioxide
CH_4	Methane
GHGs	Greenhouse gases
GWP-100	100-year global warming potentials
BD	bulk density
WFPS	Water-filled pore space

1. Introduction

Climate is undergoing significant changes, and a strongly contributing factor to this phenomenon is the release of greenhouse gases into the atmosphere. Global greenhouse gas emissions resulting from human activities has to be reduced. It is estimated and reported that atmospheric CO_2 concentrations attained 410 ppm, CH_4 1866 ppb and N_2O 332 ppb, in 2019 (IPCC 2023). Agriculture corresponds to about 50% of global CH_4 emissions and 75% of the total N_2O emissions (Tubiello et al. 2022). Greenhouse gas emissions from agriculture in Europe have been estimated at 2.1 Gt CO_2eq yr^{-1} in 2019 (Tubiello et al. 2022). There are doubts about the current IPCC guidelines for national inventories of greenhouse gases from agriculture because predicting N_2O emissions based on crop nitrogen N content is challenging (Olesen et al. 2023). The evidence presented thus far supports the idea that adaptation and mitigation responses in agriculture hold significant importance and collaboration between farmers, researchers and policy makers is critical (IPCC 2023).

Cover crops cultivated between main crops, are being proposed as a sustainable agricultural practice to abate climate change and promote long term stability in agroecosystems. This is due to the growing consensus about benefits of cover crops such as enhanced biodiversity and soil fertility, increased soil organic carbon (SOC) stocks, reduced erosion, preserved soil nutrient resources and supressed pest and diseases (Abdalla et al. 2019). However, it is not yet fully explored how different species and their residues perform under different agricultural management and climatic conditions in terms of nutrients cycling and GHG emissions (Olofsson and Ernfors 2022). This highlights the importance of building scientific knowledge about how selected cover crops and cover crop residues can alter the availability of mineral nitrogen, carbon, and oxygen and hence also the availability of nitrate NO_3^- for leaching and production of N_2O, CO_2, and CH_4 (Olofsson and Ernfors 2022). The drivers for GHG emissions in a cultivation system can be plant material related variables, environmental conditions both from soil and atmosphere and cropping system management (Olesen et al. 2023). Amidst the concurrent surge in cover crop adoption and heightened climate change awareness, there is an increasing imperative to understand these interactions.

Oilseed radish (*Raphanus sativus* L.) is a cover crop commonly cultivated by many farmers, especially in Scandinavia, where the growing season is short. It is a fast-growing species with a deep rooting system which makes it an common choice (Nilsson et al. 2024). Additionally it is a frost-sensitive crop and can be terminated by frost at different ages in field conditions. This can be due to different sowing times and variations in weather conditions between years. Olofsson and Ernfors

(2022), found that frost-killed oilseed radish increased N_2O emissions and this needs to be further researched.

The quality of cover crop residues can affect the rate of decomposition and the dynamics of N (Mary et al. 1996). Younger plant material can be expected to have a lower C:N and more of easily decomposable C compounds and these two factors have been connected to higher N_2O emissions (Lashermes et al. 2022). Environmental modifiers such as freeze-thaw are of great importance, as it is reported that freeze-thaw events can induce higher N_2O emissions, as a result of higher nitrification and denitrification rates (Mørkved et al. 2006). The purpose of this study was to find out the correlation between nitrous oxide emissions and of oilseed radish frost-killed at different ages.

1.1 Background

1.2 N_2O emissions from soils

Emissions of N_2O from soils, have been found to be of great significance in the total GHG balance of agroecosystems (Butterbach-Bahl et al. 2013; Nilson et al. 2024). Within soil, N_2O production predominantly takes place within localized regions known as ‘’hotspots’’ (Lashermes et al. 2022). These hotspots, often occupying very small soil volumes (< 1 cm^3) and exhibit short-term persistence, usually less than 2 weeks. It has been studied that ‘’hotspots’’ emerge around plant roots and residues, particulate organic matter fragments, and within soil aggregates (Kravchenko et al. 2017).

Multiple mechanisms contribute to N_2O formation within soil, as indicated by Butterbach-Bahl et al. (2013). Predominantly, N_2O production occurs via nitrification and denitrification, although the importance of emerging and under-investigated mechanisms warrants attention, as they may substantially influence N_2O emissions under certain conditions (Wrage-Mönnig et al. 2018).

Nitrification refers to the microbial oxidation of ammonia into less reduced forms, primarily nitrite (NO_2^-) and nitrate (NO_3^-) (Robertson and Groffman 2015). Furthermore, under oxygen-limited conditions, ammonium oxidizing bacteria utilize NO_2^- as an electron acceptor, resulting in the production of nitrous oxide (Firestone and Davidson 1989). Nitrification occurs through two pathways: autotrophic nitrification and heterotrophic nitrification (Robertson and Groffman 2015). Interestingly, heterotrophic nitrifiers exhibit a much higher N_2O production per cell compared to autotrophic nitrifiers (Zhang et al. 2015). Denitrification is a microbial process that occurs under anaerobic conditions. During this process, nitrate NO_3^- is sequentially reduced via NO_2^- to gaseous nitrogen oxides (NO, N_2O) and (N_2). These gaseous products are then transported through the soil and released into the atmosphere (Hansen et al. 2019). Particularly, high rates of N_2O production are more commonly associated with denitrification rather than nitrification (Firestone and Davidson 1989). Compelling evidence indicates that the denitrification process is hindered at the N_2O stage due to the malfunction of the enzyme nitrous oxide reductase (N2OR), preventing it from progressing further to N_2 (Thomson et al. 2012).

1.3 Environmental factors influencing N_2O emissions from soils

Throughout the year, various factors may impact soil N_2O emissions, and the complexity of the relationships among processes and biotic and abiotic factors is not fully understood (Butterbach-Bahl et al., 2013). Environmental factors include soil moisture, temperature, pH, nitrogen supply, and N mineralization (Butterbach-Bahl et al., 2013; Lashermes et al., 2022). N_2O emissions “hot-spots” occur, when factors, from those mentioned, occur in synchronization (Nilson et al. 2024). Production processes tend to occur in specific niches that provide the ideal conditions necessary for the particular process (Zhang et al. 2015). One factor that has been found to influence N_2O emissions is soil pH, with Zhang et al. 2015 establishing a direct correlation between an increase in soil pH and an enhanced rate of autotrophic nitrification.

When nitrogen levels are low, microbes immobilize organic nitrogen by reducing mineralization. Consequently, when carbon is scarce, N_2O production is enhanced, due to conditions that favor N_2O production pathways (Wrage-Mönnig et al. 2018). Additionally, the gross heterotrophic nitrification rate exhibits a linear increase with higher soil carbon-to-nitrogen C:N ratios. This indicates that the availability of organic substrates with elevated C:N ratios plays a pivotal role in controlling the gross heterotrophic nitrification rate (Zhang et al. 2015).

A pivotal modifier that can significantly influence N_2O emissions is the equilibrium between oxygen O_2 supply and demand (Li et al. 2016). Soil moisture plays a crucial role in determining oxygen supply, thereby affecting soil redox potential and the relative contributions of aerobic and anaerobic processes (Sylvia 1998). In this context, water-filled pore space (WFPS) has been connected to N_2O emissions, serving as a proxy for soil O_2 status and availiability to soil microbes (Duan et al. 2018; Li et al. 2016). In addition, enhanched microbial acitivity, promotes oxygen consumption (Huang et al. 2004). Notably, when soil moisture reaches approximately 60% WFPS, favorable conditions for aerobic processes (such as nitrification) arise, facilitated by unrestricted diffusion of substrates and gases O_2 (Song et al. 2019).

Freeze–thaw cycles is another environmental modifier that can contribute to nitrous oxide emissions. The probability of N_2O emissions rises during freeze-thaw (FT) cycles. It has been estimated that the emissions of nitrous oxide occurring at or shortly after thawing make up 17-28% of the worldwide N_2O emissions from agriculture (Wagner-Riddle et al. 2020). Due to climate change, it's projected that the frequency and severity of freeze-thaw cycles will rise within the temperate-boreal region (Klöffel 2024). FT cycles can increase the availability of substrates for microbes, as the FT events cause soil aggregates to break down, microbial cells death, and surviving microbes to release stored osmolytes, all of which can further drive N_2O production (Wagner-Riddle et al. 2020). N_2O can form under frozen conditions, aided by thin layers of liquid water within soil pores that maintain microbial activity. In the thawing phase, the discharge of N_2O that was previously confined beneath ice and snow is prompt (Congreves et al. 2018). Moreover, the thawing process can lead to prolonged low-oxygen conditions that is mediated by high water content, which are conducive to denitrification (Mørkved et al. 2006).

1.4 Cover crops, residues and N_2O emissions

Implementing cover crops presents multiple benefits, notably the enhancement of soil carbon sequestration and the mitigation of soil erosion through the improvement of soil's physical and biological properties. Such practices contribute to the agroecosystem. Weed suppression and pest management have been reported. This can be due to the increase in both root and soil microbial biomass, alongside a rise in beneficial insects and earthworm populations (Shu, et al. 2021). Additionally, cover crops serve as a sustainable source for bioenergy, provide fodder for livestock, and act as raw material for biorefineries (Nilson et al. 2024). In field conditions, combining reduced tillage with cover cropping can have a greater effect on ecosystem services and environmental sustainability. The selection of cover crops is influenced by a variety of factors, including the microclimate, soil characteristics, and the specific objectives of the farmer (Magdoff, F. & Van Es 2009). Cover crops are typically killed on the soil surface or incorporated into the soil before reaching full maturity. In colder climates, cover crops can also be killed naturally by frost (Magdoff, F. & Van Es 2009).
While certain research indicates that cover crops can diminish the release of the potent greenhouse gas N_2O, there is also evidence that cover crop cultivation might

lead to increased N_2O emissions (Nilson et al. 2024). The extent and pattern of these emissions are not uniform and differ across various species and conditions.

Leaving the plant residues on the surface, with minimal soil contact, has been associated with reduced N_2O emissions compared to incorporating them into the soil (Muhammad et al. 2019). The amount of N_2O released during the decomposition of cover crop biomass depends on the type of cover crop and its maturity level at termination (Singh et al. 2020). The quantity and biochemical composition (quality) of the residues in the field are among the key determinants of N_2O emissions (Li et al. 2016). Quality includes the content of carbon, and nitrogen, the ratio of C to N, the amounts of lignin, cellulose, and soluble substances, as well as the moisture level of the residues (Janz et al. 2022).

1.5 Oilseed Radish (*Raphanus sativus* L.), residues and N_2O emissions

Oilseed radish (*Raphanus Sativus* L.) is becoming increasingly popular due to its rapid growth in the later part of the year, which facilitates a substantial nutrient absorption. Studies have indicated that although other cover crop varieties might present a reduced potential for elevated N_2O emissions, oilseed radish, known for its rapid growth, produces more above and below ground biomass and emissions can be affected (Nilson et al. 2024). This plant grows a robust taproot that can reach a diameter of approximately 2.54 to 5.08 cm and extend into the soil, penetrating compacted layers and enhancing the root growth of subsequent crops. When winter comes, the oilseed radish is killed and decomposes by the time spring arrives, leaving the soil loose and crumbly with holes from the roots that help with water absorption and retention (Magdoff, F. & Van Es 2009).

Nevertheless, during winter's freeze-thaw cycles, there's an increased likelihood of higher nitrous oxide (N_2O) emissions from oilseed radish. This is due to the release of nitrogen and carbon from cover crop biomass into the soil, which occurs under conditions that promote denitrification (Olofsson and Ernfors, 2022).

2.Aim and Hypotheses

The aim was to elucidate the relationship between N_2O emissions and different ages, of immature frost-killed oilseed radish, assuming that cover crop age affects the quality of the cover crop aboveground biomass. The research questions addressed were: 1. Does oilseed radish frost-killed at different ages has different N_2O emissions? 2. How does the quality of the oilseed radish frost- killed at three different ages affect the N_2O emissions?

The following hypotheses were tested to help answering the research questions:
1. Oilseed radish frost-killed at younger ages results in higher N_2O emissions compared to when it is frost-killed at older ages.
2. Younger oilseed radish decomposes more easily, releasing CO_2 faster than older material.

3. Materials and Methods

3.1 Experimental setup

A mesocosm incubation study was carried out, with field soil and oilseed radish aboveground biomass of different ages, over 7 days. Both soil and plant material were frozen and thawed just before the start of the incubation, to mimick a freeze-thaw episode killing an oilseed radish cover crop. During incubation, treatments were stored in a dark cabinet at 19 °C for 7 days, to take gas samples and analyse for N_2O, CO_2 and CH_4 fluxes.

The study included four treatments, which consisted of cylinders with only soil as control and soil with oilseed radish frost-killed at 3 different ages:

1. Age 1 (21 days): The plant material used in the soil cylinders was grown for 21 days (10.02.2024 -01.03.2024).
2. Age 2 (28 days): The plant material used in the soil cylinders was grown for 28 days (10.02.2024 -01.03.2024).
3. Age 3 (35 days): The plant material used in the soil cylinders was grown for 35 days (10.02.2024-08.03.2024).
4. Csoil: Control soil cylinders without any added plant material.

The treatments were arranged in a randomized block design, and each treatment was replicated four times.

3.2 Soil sampling

The soil used in the cultivation and the experiment was collected from SITES Lönnstorp Research Station, located in southern Sweden (latitude 55.67ºN 13.11ºE). Samples were obtained from the 0-20cm layer of a bare field that had previously been cultivated with barley *Hordeum vulgare* L. It is classified as a loamy with 22% clay, 22% silt and 3% organic material.

3.3 Oilseed radish *Raphanus Sativus* L. cultivation

A first step in conducting the experiment was the cultivation of oilseed radish, for use in the incubation. Oilseed radish was cultivated in the greenhouse. Temperature was modified at 20°C during the day and 18°C during the night. Greenhouse ventilation was justified at 22°C during the day and 20°C during the night with an ambient relative humidity set at 65%. Supplementary artificial light was provided from 8:00- 19:00 with high pressure sodium lamps. Oilseed radish seeds (3.93g) were sown in 13.4 L pots using soil as a growing medium, replicating field conditions. The soil used was collected a day within a rainy period, resulting in a high water content. To prepare it for cultivation, the soil was first air-dried at room temperature for a period of 5 days to reduce the moisture content. Following the drying period, the soil was then subjected to crushing, using a mortar and pestle to decrease the soil particles size. The seeds were sown at a depth of 2cm and the pots were immediately irrigated. The plants were monitored daily throughout the cultivation period (35 days, 2024.02.10- 2024.03.18), receiving irrigation as required. No fertilizers were applied, which is common practice for oilseed radish and other cover crops. Additionaly, a potential impact on the nitrogen balance, as well as economic and environemtal factors, were taken into account.

3.4 Aboveground biomass preparation and analyses

Oilseed radish was harvested at three different ages, with the aboveground biomass being cut 50 mm above the topsoil to prevent soil contamination. The three different ages of oilseed radish mentioned throughout the thesis, refer to plant material harvested on three specific dates. Age 1 corresponds to plants grown for 21 days and harvested on the 21st day of the cultivation period (10.02.2024 -01.03.2024) . Age 2 refers to plants grown for 28 days and harvested on the 28th day (10.02.2024-08.03.2024) , while Age 3 plants grown for 35 days and harvested on the 35th day (10.02.2024-15.03.2024).

The aboveground biomass of oilseed radish frost-killed at 3 different ages, used for the incubation experiment was prepared immediately, after each harvest. After each harvest, leaves were manually mixed on a clean, flat surface. Four subsamples from each treatment were then randomly selected, weighed (6.7g) and packed in zip-lock plastic bags. Then these subsamples were placed in a freezer at -27°C and kept until 05.04.2024, when the incubation started (day 1).

Subsamples of oilseed radish from each harvest were obtained for calculation of DM content and Total C, Total N analyses. For DM content estimation, the plant biomass was weighed with a Mettler Toledo ME4002 balance, then dried at 60°C overnight, and weighed again. For the analyses of Total C and Total N, two representative subsamples were obtained from the total dry weight. Firstly subsamples were grinded for homogenization in a Retsch Mixer Mill MM 400. The frequency of grinding was set at 30 1/s (30 cycles per second) for 2 seconds. Post-grinding, the subsamples were subjected to a further 2-hour drying period in the oven at 60°C. A precise weight of 6mg was measured for each subsample using a Mettler Toledo XP6 Micro Balance. The final stage involved the analyses of Total N and Total C content, which were performed in a Thermo Scientific FLASH 2000 Analyzer, utilizing Helium as the carrier gas.

3.5 Soil preparation and analyses

The soil, initially wet upon collection, was evenly distributed and allowed to air-dry at ambient room temperature to achieve a moisture content conducive for subsequent sieving. Subsamples of the soil were then taken for the purpose of analyzing Total Carbon, Total Nitrogen, and for pH measurement. The soil was crushed using a mortar and pestle, followed by sieving to obtain particles of less than 6 mm in size. The remaining soil, set aside for the incubation experiment, was stored in a plastic bag and placed in a cold storage room to preserve its physico-chemical and biological properties until further use.

For pH measurement, the 1:2 ratio soil-to-water extract procedure was conducted. A sample of air-dried soil (15g) was weighed, and deionized water (30g) was added in a glass beaker. Then, the mixture was placed on a mechanical shaker for 2 hours. Afterward, the pH was measured with a Fisherbrand accumet AB150 pH benchtop meter, using the tip of the electrode.

Subsamples of soil from were obtained for Total C, Total N analyses. While the general procedure is consistent with that described in section 4.4 for plant material, the notable deviation is the sample mass; for soil, the mass was adjusted to 30 mg, in contrast to the 5 mg used for plant material.

3.6 Preparation for incubation

Soil was treated and prepared as described in section 4.5. For the packing of soil in the cylinders soil water content was determined gravimetrically by oven drying soil samples (100g) at 105 °C overnight. The soil was mixed with of water by spraying to achieve approximetaly WFPS 60% based on the soil bulk density of 1.25 g cm^{-3}. Drying and wetting of the soil could possibly affect the N balance and consequently the emissions, but for the soil used for the incubation, the soil was mixed well and subsamples for each cylinder were obtained in order to avoid differences.

Cylinders were prepared by stepwise packing of 370 g soil in 4 layers, each 2cm thick, to reach a total height of 6cm. Following that deionised water was added to the soil surface with a syringe with a needle 0.4mm to reach 60% WFPS. The top and the bottom of each cylinder was covered with Parafilm. The Parafilm on the top of each cylinder was pierced with 10 holes using a 0.8 mm needle. After packing the soil into the cylinders, they were left for half an hour before freezing to allow the water content to equilibrate. The soil cylinders were then placed in a freezer (-27°C).

3.7 Gas sampling and analysis

Before 1 hour of the first measurement (morning- T_0 time) on day 1 sampling, plant material samples and soil cylinders, were removed from the freezer and top Parafilm was removed. Packing of soil with frost-killed oilseed radish in the three different ages was done. The plant residues were added on the soil surface of each labelled cylinder, and finally, 5ml of water were added by a syringe with a needle 0.4 mm in the cylinder to simulate the conditions of thaw events in the field. The cylinders were again covered with Parafilm that was pierced with 10 holes using a 0.8 mm needle. After jars, cylinders and equipment were ready the gas sampling took place in a sampling table.

Gas sampling took place on days 1, 2, 3, 4, 5, and 7. On days 1, 2, and 3, samples were collected twice daily: once in the morning and once in the afternoon. Two samples were collected for each measurement- one at T_0 time and another at T_{80} time.

Firstly glass jar no.1 was placed in the sampling table and two wooden sticks were placed to prevent the soil sample from sticking to the bottom of the jar. After the cylinder (without the top Parafilm) has been placed in the jar, the lid was closed. Before each measurement, a syringe used for sampling, was ‘’aired’’ three times. A hypodermic needle (0.4 mm) of the syringe was then inserted through the rubber septum in the center of the jar lid. The syringe was filled to 9 ml, the stopcock was closed, and the sample was transferred to a pre-evacuated Exetainer vial. After sampling, the syringe was again “aired” three times by pulling out to 10 ml. This procedure was repeated for all 16 different treatments.

After 35 minutes, the T_{80} measurement was performed using the same process. Following each measurement in each glass jar, the cylinder was removed, sealed with Parafilm (pierced with 10 holes using a 0.8 mm needle), and placed in the incubation cabinet until the next T_0 measurement.

The concentrations of N_2O, CO_2, and CH_4 were quantified through gas chromatographic analysis. This was performed using an Agilent 7890A Gas Chromatograph.

3.8 Calculations and statistical analyses

Gas flux calculations for all greenhouse gases were conducted based on the dry matter of added plant material in the relevant treatment cylinders. Additionally, fluxes were calculated per area for all cylinders across treatments

For the calculation of each greenhouse gas flux based on Ideal Gas Law, the following parameters were used: actual temperature of 19°C, actual pressure of 1000 Pa (data from SITES, SLU), jar closure time of 1.33 hours, jar volume of 1154 cm^3, volume of steel (cylinders) and wood (wooden sticks) at 20 cm^3, volume of dry soil in the cylinders at 40 cm^3, volume of water in the cylinders at 37 cm^3, net jar volume of 1000 cm^3, core area of 0.004 m^2, and the volume of the gas sample at 9 cm^3. Additionally, T_0 and T_{80} values (in ppm) from the analysis were used for each gas calculation. The underpressure caused by the collection of the first sample was compensated for in the calculation for the second sample. The fluxes were integrated over the 6 day period using linear interpolation between the measurement points.

After calculations, statistical analyses conducted. For the statistical analyses, univariate general linear model and post hoc Tukey test in SPSS software were used (IBM Corp. Released 2024. IBM SPSS Statistics for Windows, Version 29.0.2. Armonk, NY: IBM Corp). A significance level of $p < 0.05$ was set. To address the normality and homoscedasticity assumptions, transformations where applied when needed on both data sets. This transformation helps stabilize variance and achieve a more symmetric distribution, to make sure that the data meets the criteria for the statistical test to be valid.

Mean cumulative emissions of N_2O and CH_4 from each treatment were converted into carbon dioxide equivalents (CO_2-eq) using the 100-year global warming potentials GWP-100. This metric has been extensively employed in climate policy to standardize the reporting of emissions from different greenhouse gases (GHGs) on a comparable scale from (Forster et al. 2021).

4. Results

4.1 C and N content of oilseed radish

Total Carbon C_{tot} values that represent the amount of carbon present in the oilseed radish in each different ages, Total Nitrogen N_{tot} values for the nitrogen content and the carbon-to-nitrogen C:N ratio are presented in Table 1. Although no statistical analysis was carried out, since there were no replicates, the data indicate an increase in total carbon content as the oilseed radish develops in age, with measurements of 31.6 % at Age 1, 34.6 % at Age 2, and 41.8 % at Age 3. Conversely, total nitrogen content does not exhibit a consistent pattern with oilseed radish age, with values recorded at 6 for Age 1, 5.5 for Age 2, and a decrease to 2.3 for Age 3. Furthermore, the C:N ratio demonstrates an increase with plant age, from a ratio of 6 at Age 1 to 6.3 at Age 2, and a marked rise to 18 at Age 3.

Table 3. Oilseed radish quality parameters. Ctot, Ntot, C/N ratio oilseed radish in three different ages.

	Age 1 (21 days)	**Age 2 (28 days)**	**Age 3 (35 days)**
C_{tot} (%)	31.6	34.6	41.8
N_{tot} (%)	6	5.5	2.3
C:N ratio	6	6.3	18

4.2 Environmental modifiers

Environmental modifiers such as the air temperature recorded during the experiment, the soil pH, and the bulk density, which was measured in the soil used for both cultivation and incubation are presented in Table 2. It also details the total carbon C_{tot} and total nitrogen N_{tot} contents of the soil, alongside the soil C:N ratio. Additionally, the value of initial WFPS is provided for the duration of the incubation.

Table 4. Air temperature, soil pH, bulk density, WFPS, soil Ctot, soil Ntot, soil C/N ratio.

Air temperature (°C)	19
soil pH	6.9
Bulk density (g cm^{-3})	1.25
WFPS (%)	60%
soil C_{tot} (%)	2.4
soil N_{tot} (%)	0.2
soil C:N ratio	11.4

4.3 N_2O emissions

The results from statistics with data used from the calculations of N_2O per dry weight of added plant material were significantly different as shown in Figure 1a. Age 1 (21 days) accounted for 2533 µg N_2O-N g DM added^{-1} period^{-1} , appear to be significantly higher from Age 2 (28 days) with 231 µg N_2O-N g DM added^{-1} period^{-1} and Age 3 (35 days) with 331 µg N_2O-N g DM added^{-1} period^{-1}. N_2O fluxes per dry weight (µg N_2O-N g DM added^{-1} period^{-1}) throughout the full study period and the different hours of measurements are depicted in Fig. 2.a.

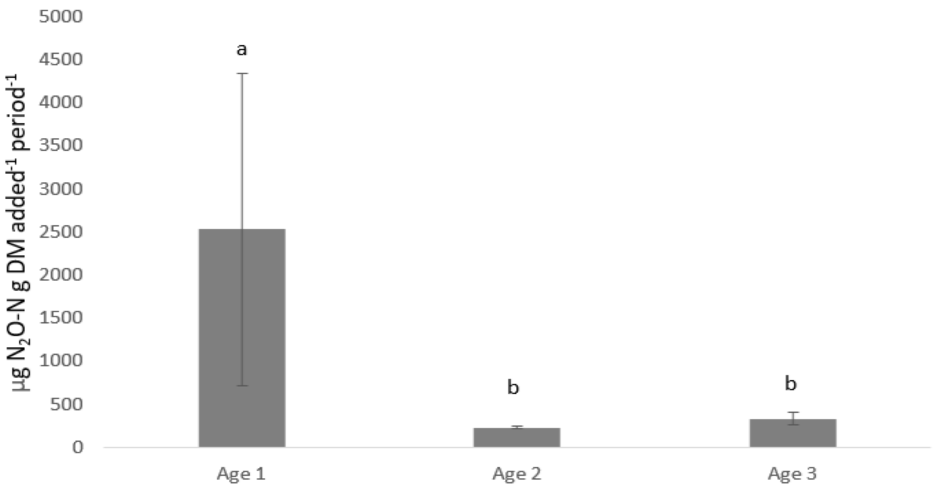

Figure 1.a. Cumulative emissions of N_2O per dry weight (µg N_2O-N g DM added^{-1} period^{-1}) is the N_2O-N evolved per dry weight of added oilseed radish for the cylinders where plant material was added, during the full study period. Period accounts for the 6 days of measurements. Age 1 (21 days), Age 2 (28 days), Age 3 (35 days). Standard error (SE) is represented with the error bars. The letters (a, b) indicate significant differences between treatments.

The results from the data from N_2O flux per area revealed that the treatments with added oilseed radish were all higher than the control treatment. This is reflected in the F value of 12.774, which exceeds the threshold for statistical significance, and a p value of less than .001. No differences among the treatments were indicated.

Mean cumulative emissions of N_2O per area (N_2O-N g ha^{-1} d^{-1}) at each different treatment during the full study period are presented in Fig.1.b. and N_2O fluxes per area throughout the full study period in Fig.2.b. During the 6-day measurement period, we observed significant peaks in emissions associated with the Age 1 treatment within Block A. When expressed as carbon dioxide equivalents (CO_2-eq.), the mean cumulative emissions were as follows: 686.2 CO_2-eq. for Age 1 (21 days), 120.4 CO_2-eq. for Age 2 (28 days), 204.9 CO_2-eq. for Age 3 (35 days), and 18.3 CO_2-eq. for Csoil.

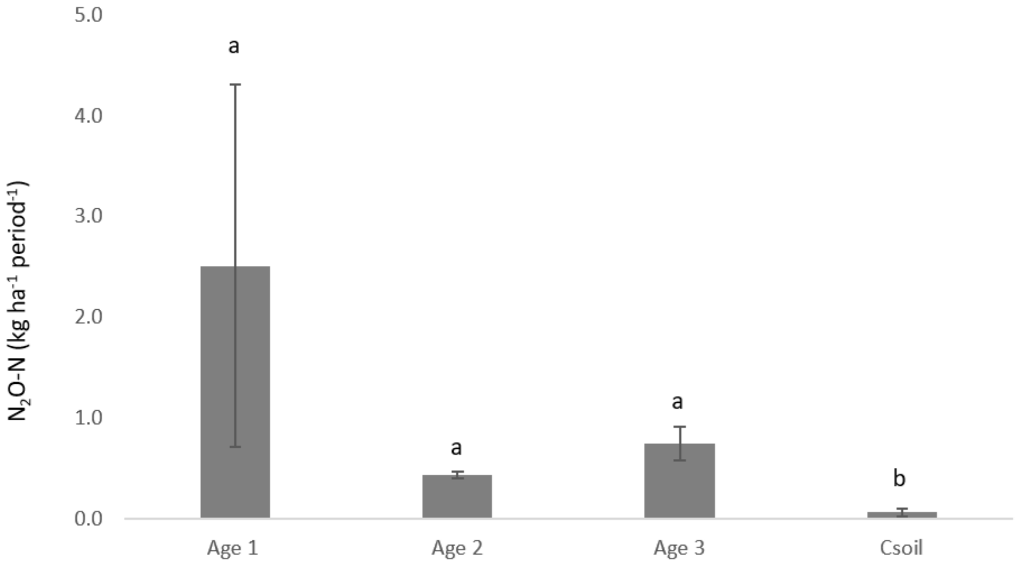

Figure 1.b. Mean cumulative emissions of N_2O per area (N_2O-N g ha^{-1} d^{-1}) at each different treatment during the full study period. Period accounts for the 6 days of measurements. Age 1 (21 days), Age 2 (28 days), Age 3 (35 days), Csoil (only soil). The error bars represent standard error (SE). Different letters (a, b) indicate significant differences between treatments.

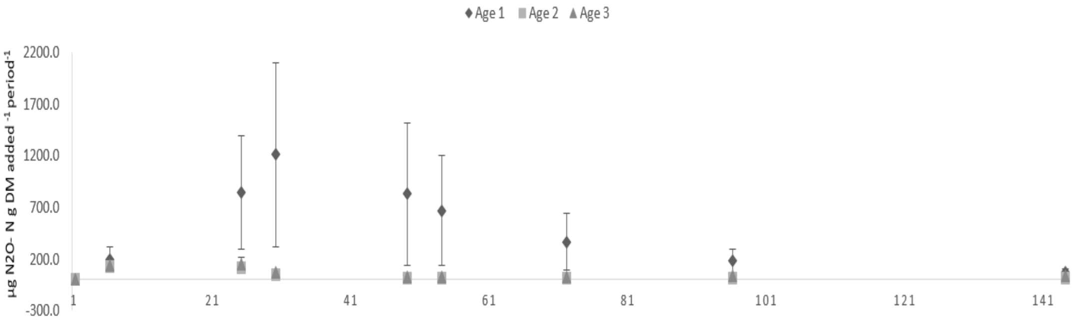

Figure 2.a. N_2O fluxes per dry weight (µg N_2O-N g DM added^{-1} period^{-1}) throughout the full study period. Period accounts for the 6 days of measurements. In the horizontal axis hours of measurements are presented. Age 1 (21 days). Age 2 (28 days), Age 3 (35 days). The error bars represent standard error (SE).

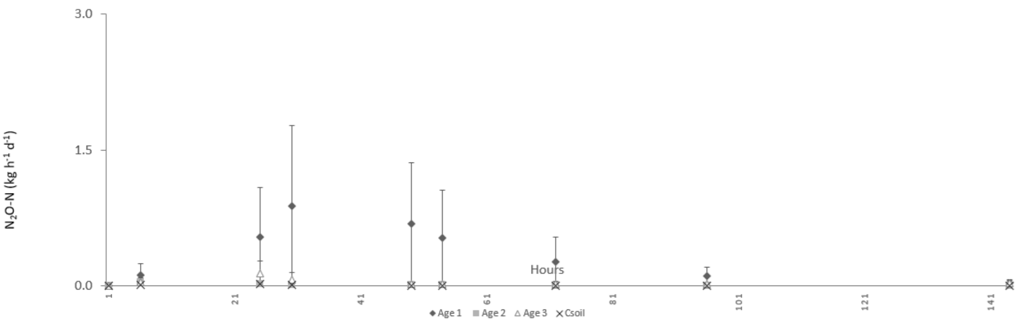

Figure 2.b. N_2O fluxes per area (N_2O-N g ha^{-1} d^{-1}). Age 1 (21 days), Age 2 (28 days), Age 3 (35 days), Csoil (only soil). The error bars represent standard error (SE).

4.4 CO_2 and CH_4 emissions

The results from statistics with data used from the calculations of CO_2 emissions per dry weight of added plant material showed significant differences as shown in Figure 3.a. Age 1 (21 days) were higher CO_2 from Age 2 (28 days) and Age 3 (35 days). Age 2 (28 days) fluxes were higher than Age 3 (35 days). CO_2 fluxes per dry matter (mg CO_2-C g DM added^{-1} period^{-1}) throughout the full study period, with values corresponding to the hours of measurements are depicted in Fig.4.a.

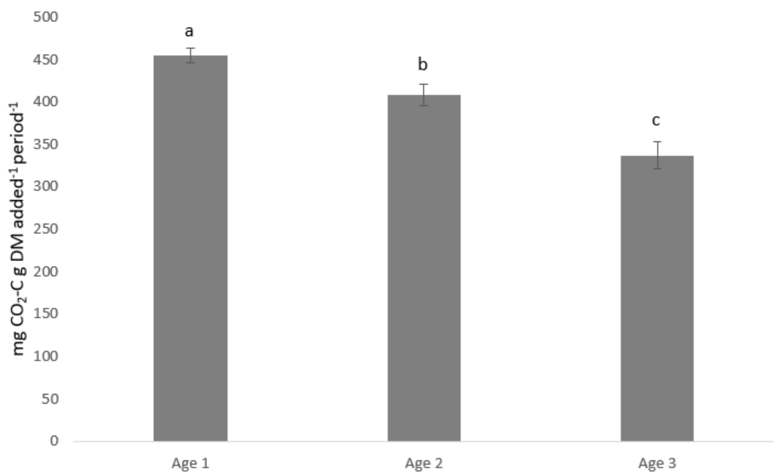

Figure 3.a. Cumulative emissions of CO_2 per dry weight (mg CO_2- C g DM added^{-1} period^{-1}) is the CO_2-C evolved per dry weight of added oilseed radish for the cylinders where plant material was added, during the full study period. Period accounts for the 6 days of measurements. Age 1 (21 days), Age 2 (28 days), Age 3 (35 days). The error bars represent standard error (SE). Different letters (a, b) indicate significant differences between treatments.

The results from the data from gas fluxes per area and the mean values of CO_2-C emissions (kg CO_2-C/ha^{-1}/period^{-1}) for each treatment are presented in Fig.3.b. CO_2 levels across all age treatments Age 1 (21 days), Age 2 (28 days), and Age 3 (35 days) exhibit significant differences when compared to control treatments Csoil ($p < 0.001$). CO_2 fluxes (kg ha^{-1} d^{-1}) throughout the full study period are shown in Fig.4.b. During the 6-day measurement period, there was a consistent trend with high emissions, and peak levels occurring on the 3rd, 4th, and 5th days for Age 2 (28 days) and Age 3 (35 days).

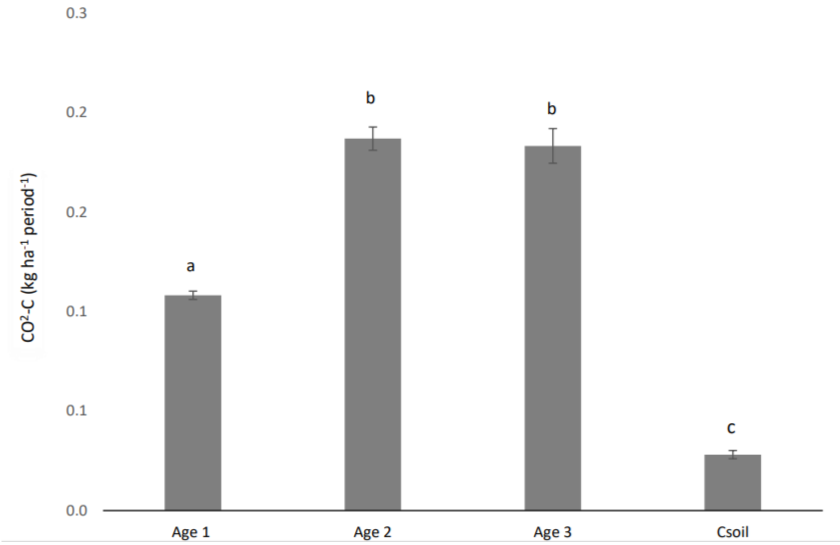

Figure 3.b. Mean cumulative emissions of CO_2 per area (kg ha^{-1} $period^{-1}$) at each different treatment during the full study period. Period accounts for the 6 days of measurements. Age 1 (21 days), Age 2 (28 days), Age 3 (35 days), Csoil (only soil). The error bars represent standard error (SE). Different letters (a, b) indicate significant differences between treatments.

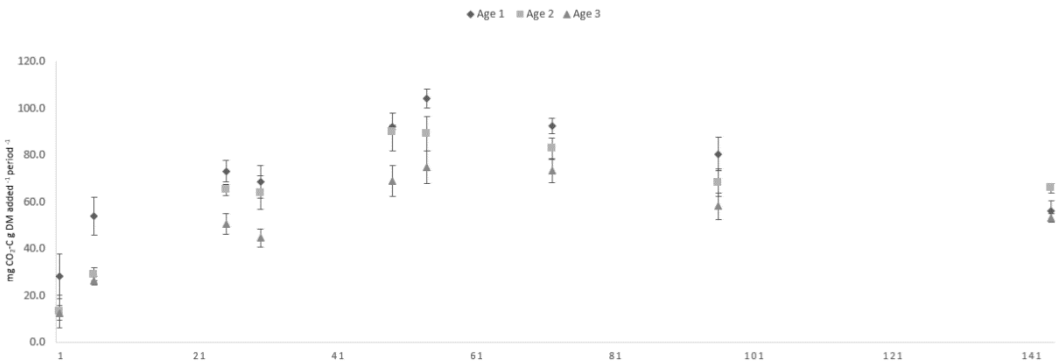

Figure 4.a. CO_2 fluxes per dry weight (mg CO_2- C g DM $added^{-1}$ $period^{-1}$) throughout the full study period. Period accounts for the 6 days of measurements. Age 1 (21 days), Age 2 (28 days), Age 3 (35 days), Csoil (only soil). The error bars represent standard error (SE).

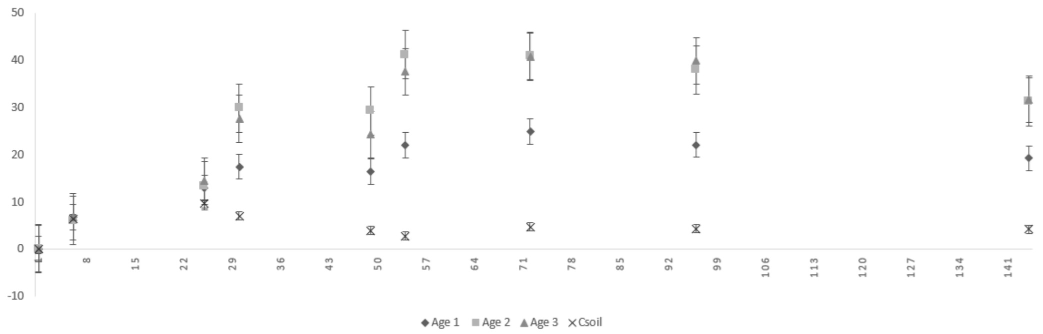

Figure 4.b. CO_2 fluxes per area (kg ha^{-1} d^{-1}). Age 1 (21 days), Age 2 (28 days), Age 3 (35 days), Csoil (only soil). The error bars represent standard error (SE). Different letters (a, b) indicate significant differences between treatments.

The data for mean cumulative CH_4 emissions per dry weight of added plant material and CH_4 fluxes per area in all cylinders revealed no significant differences among the different age groups and treatments, accordingly. Results are shown in Figure 5.a. and Figure 5.b.

CH_4 fluxes per dry weight are presented in Fig. 6.a. Emissions of CH_4 per area (kg ha^{-1} d^{-1}) throughout the full study period are presented in Fig.6.b. CH_4 emissions per area indicated some peaks without significant differences for the treatment. When expressed as carbon dioxide equivalents (CO_2-eq.), the mean cumulative emissions or uptake values were as follows: -22.8 CO_2-eq. for Age 1 (21 days), indicating uptake; 50.8 CO_2-eq. for Age 2 (28 days); 85.2 CO_2-eq. for Age 3 (35 days); and -363.7 CO_2-eq. for Csoil.

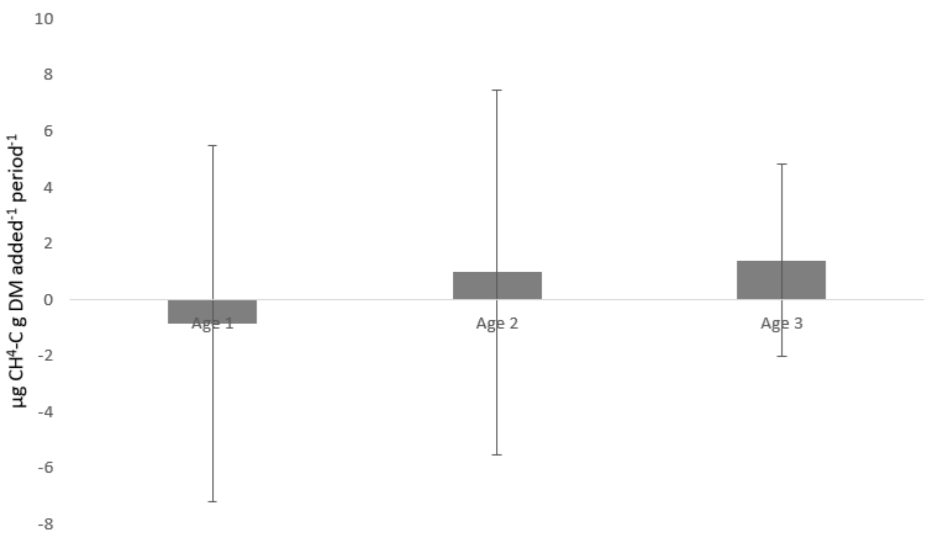

Figure 5.a. Mean cumulative emissions of CH_4 per dry matter (mg CH_4- C g DM added^{-1} period^{-1}) is the CH_4-C evolved per dry weight of added oilseed radish for the cylinders where plant material was added, during the

full study period. Period accounts for the 6 days of measurements. Age 1 (21 days). Age 2 (28 days), Age 3 (35 days). The error bars represent standard error (SE). Different letters (a, b) indicate significant differences between treatments.

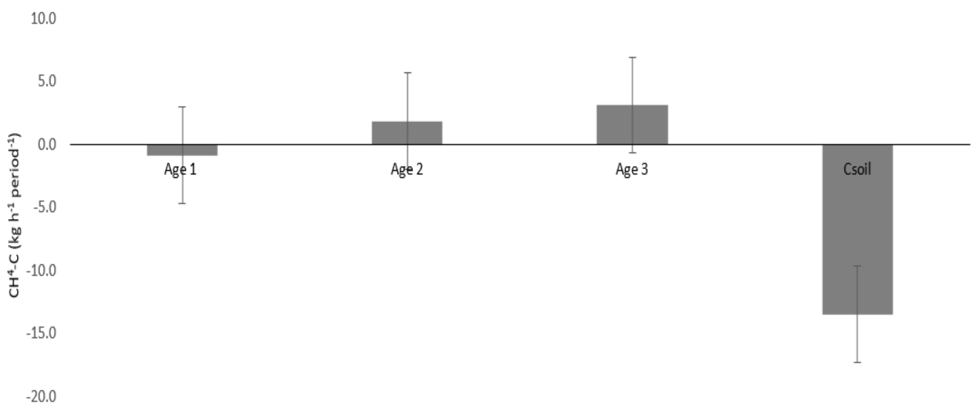

Figure 5.b. Mean cumulative emissions of CH_4 per area at each different treatment during the full study period. Period accounts for the 6 days of measurements. Age 1 (21 days), Age 2 (28 days), Age 3 (35 days), Csoil (only soil). The error bars represent standard error (SE). Different letters (a, b) indicate significant differences between treatments.

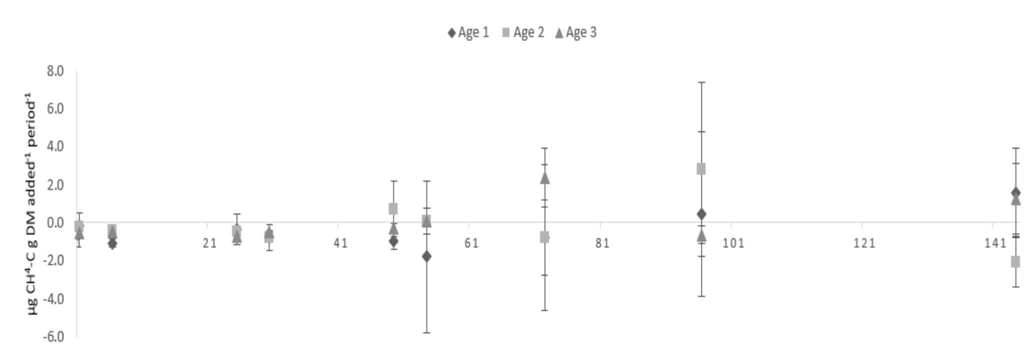

Figure 6.a. CH_4 fluxes per dry weight (µg CH_4-C g DM added^{-1} period^{-1}) throughout the full study period. Period accounts for the 6 days of measurements. Age 1 (21 days), Age 2 (28 days), Age 3 (35 days). The error bars represent standard error (SE).

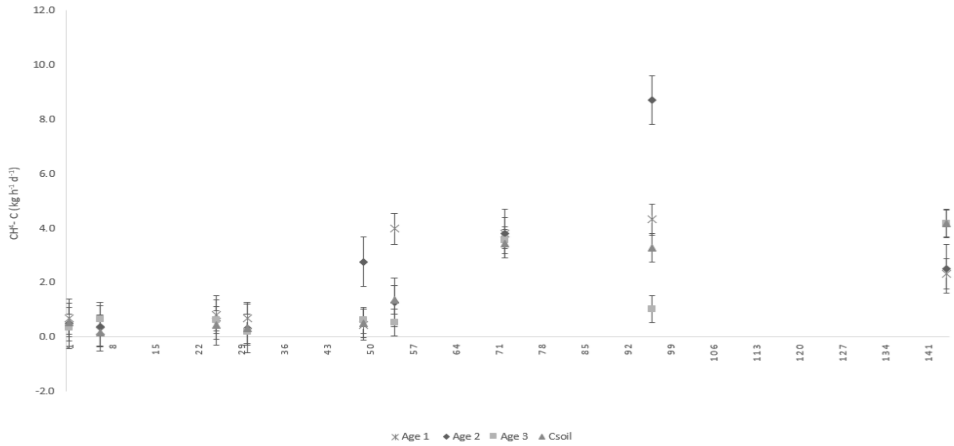

Figure 6.b. Emissions of CH_4 per area (kg ha^{-1} d^{-1}). Age 1 (21 days), Age 2 (28 days), Age 3 (35 days), Csoil (only soil). The error bars represent standard error (SE).

5. Discussion

The results presented above confirmed hypothesis 1, as evidence demonstrates a decrease in N_2O emissions with increasing age. Hypothesis 2, is confirmed as the decomposability of oilseed radish decreased with age. In the experiment, adding an equal fresh weight of plant material to each cylinder did not clearly reveal differences among the treatments based on carbon C and nitrogen N content. However, calculating the gas flux values based on the dry weight of the added plant material provided a representation of the treatment effects due to variations in the addition of C and N in the different ages.

5.1 N_2O emission magnitudes

The observed increase in the C/N ratio with oilseed radish age aligns with findings from another study (Zhang et al. 2013). Despite the relatively short intervals between Age 1 (21 days) and Age 2 (28 days), as well as between Age 2 (28 days) and Age 3 (35 days) with only 7 days between harvests, the trend remains consistent. The findings from N_2O fluxes per dry weight, show that N_2O emissions from oilseed radish frost-killed were notably higher in Age 1 (21 days). Age 1 (21 days) had the lowest C:N ratio (6) from the treatments in the experiment and significantly high emissions. Even though Age 2 (28 days) had a lower C:N ratio (6.3) from Age 3 (25 days) (18), exhibit lower N_2O emissions per dry weight in this experiment. This finding about the magnitude of N_2O diverges from previous research. It suggests that there is a nonlinear relationship between emissions and quality factors studied. Thus further investigation is proposed in order to validate the results of the current experiment. Age 1 (21 days) had lower C:N ratio and significantly higher emissions from Age 2 and from Age 3 (35 days).

According to the results per fresh weight, N_2O emissions from oilseed radish were higher across all treatments, from the control group. This result is likely to be expected for other immature cover crops as well. Furthermore, this rise in N_2O emissions when compared to Csoil aligns with previous research, that supports that the addition of plant material with low C/N ratio increases N_2O emissions (Olofsson and Ernfors 2022; Lashermes et al. 2022; Janz et al. 2022). Olofsson and Ernfors (2022) found that the frost-sensitive oilseed radish can contribute to higher emissions and a possible explanation could be its release of more decomposable carbon, in contrast to other cover crops.

5.2 Explanations of N_2O emission patterns

N_2O emissions from frost-killed oilseed radish may result from 'C effects,' i.e., the promotion of heterotrophic denitrifier activity by the addition of degradable C substrate. Additionally, C mineralization can affect soil oxygen consumption, which promotes denitrification (Olesen et al. 2023). CO_2 emissions is a measure of microbial acitivity. Based on the CO_2 emissions shown in Figure 3.a., the rate of decomposition is highest in the Age 1 (21 days) oilseed radish, as indicated by the higher CO_2 emissions. This is followed by the Age 2 (28 days) and Age 3 (35 days) oilseed radish. This decomposition process can lead to an increase of the soil microbial biomass, thereby increasing net nitrogen mineralization (Zhao et al. 2018). Subsequently production of NH_4^+ can potentially increase the rates of nitrification and denitrification (Olesen et al. 2023).

The C/N ratios observed remained below the threshold of 30, a value delineated by e.g. Chen et al. (2013) as critical. Aligning with the insights by Chen et al. (2013) and Janz et al. (2022), the results confirm the basis that plant residues with a C:N ratio lower than 30 increase N_2O emissions. In the short term mineralizable N and degradable C can affect emissions. Higher N_2O emissions observed in Age 1 (21 days), that was the early Age treatment that the oilseed radish was frost-killed. This was related with the lowest C/N ratio of the oilseed radish at that Age. CO_2 emissions recorded at Age 1 (21 days) showed that the heterotrophic microbial communities were more active in that Age, that can also explain the higher emissions. C:N ratio did not provide full explanation of the results.

Factors are consider part of the quality of the oilseed radish, such as concentration of lignin, cellulose and solutes haven't been examined due to time constraints. Determining the initial biochemical composition could enhance the results, as it has been found that the inverse relationship between crop age and N_2O emissions is due to the increased C/N ratio and lignin content in the plant biomass as the crop matures. The heightened presence of lignin is known to impede nitrogen mineralization by fostering the synthesis of polyphenols and this process potentially augments the quantity of recalcitrant nitrogen through the formation of humic polymers (Singh et al. 2020).

The production of N_2O can also be influenced by environmental variables, although kept constant in this study. Soil pH can influence N_2O emissions. The pH of the soil used for incubation was close to neutral at 6.9 that possibly affected the results. In line with previous research, when pH is above the 6.4–6.8 range considered effective for reducing N_2O emissions (Hénault et al. 2019). In addition, it has been indicated that denitrification occurring in soils with pH values below 7 results in a major product: N_2O. This is due to the fact that the reduction of N_2O to N_2 can be inhibited (Šimek and Cooper 2002).

It has been suggested that freeze-thaw cycles may elevate N_2O/N_2 product ratios. This is due to the release of substrates for ammonia-oxidizing bacteria, which produce nitrate through nitrification. The nitrate then serves as an input for denitrification and heterotrophic activity, leading to increased denitrification and oxygen consumption, ultimately affecting the N_2O/N_2 ratio during denitrification (Mørkved, P.T., et al., 2006). In exploring the empirical relationship between N_2O emissions in different ages of oilseed radish and freeze-thaw events, it is imperative to consider a range of factors that may exert significant influence especially in field conditions. Among these, cumulative freezing degree days (FDD), which quantify the cumulative cold exposure over time, serve as a critical indicator of the intensity and duration of freezing conditions. Similarly, degree-days with soil temperatures at 5 cm depth below 0°C provide a more localized measure of soil freeze dynamics, directly affecting microbial activity and gas fluxes. In addition, the concentrations of soil nitrate (NO_3^-) are important (Wagner-Riddle et al. 2017). It has been indicated that the temperature at which soils freeze may also affect N_2O emissions, with higher emissions at lower temperatures (Risk et al. 2013). In this experiment, oilseed radish samples of different ages were frozen for varying lengths of time: Age 1 (21 days) was frozen for 36 days, Age 2 (28 days) was frozen for 29 days, and Age 3 (35 days) was frozen for 22 days. Oilseed radish treatments were subjected to varying durations of freezing, while all other factors known to impact N_2O emissions during freeze- thaw cycles were kept constant. The duration of freezing conditions plays a crucial role in influencing N_2O emissions, with a positive linear relationship observed between the length of freezing periods and the magnitude of emissions (Risk et al. 2013). In the context of the current experiment, the Age 1 (21 days) from the results per dry weight, which experienced the longest freezing duration, exhibited the highest N_2O emissions. This observation aligns with existing literature that suggests a positive linear relationship between freezing duration and N_2O emissions (Risk et al., 2013). However, the N_2O emissions observed in the Age 2 (28 days) and Age 3 (35 days) treatments from the results per dry weight did not follow this expected trend. These discrepancies suggest that other factors may be influencing N_2O emissions in these treatments, warranting further investigation.

5.3 Climate impact of N_2O and CH_4 emissions

The climate impact can be estimated for both N_2O and CH_4. It is important to note that these two gases are not significantly taken up by the soil-plant system. Therefore, it is relevant to estimate their climate impact even if they are measured over a short period. N_2O global warming potential GWP-100 is 273 times that of CO_2 (IPPC 2021). Positive cumulative emissions values (e.g., 686.2 CO_2-eq. for Age 1, 120.4 CO_2-eq. for Age 2 (28 days), 204.9 CO_2-eq. for Age 3 (35 days), and 18.3 CO_2-eq. for Csoil) indicate N_2O release, contributing significantly to climate change, even in short- term.

CH_4 emissions appear not to have high values throughout the period of gas measurements and no differences between the treatments were indicated. If there were differences, CH_4 emissions could have been used as an indicator for low oxygen availiability and thus could be helpful in explaining differences in N_2O emissions, but this is not the case for these results. CH_4 has a GWP-100 is 27 times that of CO_2, making it impactful in the short term, but less that N_2O. Negative cumulative values (e.g., -22.8 CO_2-eq. for Age 1 (21 days) and -363.7 CO_2-eq. for Csoil) suggest CH_4 uptake. In contrast, there are positive values for Age 2 (28 days) accounting for 50.8 CO_2-eq. and for Age 3 (35 days) accounting for 85.2 CO_2-eq.

In contrast, CO_2 emissions during a certain period may be counteracted by photosynthetic uptake during another period of the year on the same piece of land in field conditions (Vicca 2018). Consequently, only net CO_2 emissions over longer periods, such as a year or several years, are relevant for assessing the overall climate effect.

6.Conclusions

The results from the data of N_2O per dry weight, showed that oilseed radish frost-killed at different ages produced varying levels of N_2O emissions. Notably, Age 1(21 days) that was the younger plant material showed higher N_2O emissions. Age 2 (28 days) and Age 3(35 days) showed lower N_2O emissions. In addition the findings confirmed that the addition of frost-killed immature oilseed radish aboveground biomass in soil with relatively high water content increases N_2O emissions. Furthemore, the study demonstrated that the decomposability of oilseed radish decreases with plant age, even over short time periods. This was evidenced by the microbial activity induced by the different treatments, as shown by the CO_2 data per dry weight. Specifically, the Age 1(21 days) oilseed radish was the most easily decomposable, significantly increasing microbial activity, followed by Age 2(28 days) and Age 3(35 days) oilseed radish. From this thesis, the conclusion can be drawn that in field conditions, N_2O emissions from frost-killed oilseed radish, and potentially from oilseed radish terminated by other methods, could be lower if the oilseed radish is sown earlier as a cover crop.

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Popular science summary

Climate change and research is undergoing in order to find ways to mitigate impacts that agricultural practices may have. Agriculture plays a significant role in greenhouse gas (GHG) emissions. Efforts for adaptation and mitigation are crucial.

Cover crops, are cultivated for soil health and other ecosystem services. However, some of these crops and what is left in the field can also release a gas called nitrous oxide, which contributes to global warming. Some of them can decrease emissions. As a component of sustainable agricultural practices, cover crops deserve comprehensive evaluation. Additionally, the way we manage cover crop residues significantly influences their impact on the environment and subsequent crop in the field. Furthermore, considering the effects of climate change and weather fluctuations is essential.

Oilseed radish (*Raphanus sativus* var. oleiformis), valued for its ecosystem services and economic viability as a cover crop, especially in Scandinavia, warrants attention. Recent studies suggest that the assessment of nitrous oxide N_2O emissions is crucial. This consideration ensures a balanced understanding of both the beneficial and adverse environmental impacts, thereby empowering farmers with informed decision-making capabilities and help them increase Nitrogen Use Efficiency (NUE), reduce their inputs and support the environment.

The objective of this thesis was to explore the relationship between the emissions of nitrous oxide N_2O and the different ages of oilseed radish frost-killed. Oilseed radish is a cover crop that can be terminated by frost in the field, even when the exact termination dates are uncertain due to weather fluctuations.

The findings of this study add to existing knowledge by demonstrating that oilseed radish cultivated as a cover crop, when killed by frost at different ages, can lead to high emissions. The results showed a surprising value for the younger one ‘’Age 1’’ and a relationship with the C and N added from this plant material.

Further investigation and validation of the results is proposed.

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